T H E S I S

Presented for the Degree of Ph.D.

Edinburgh University.

# Studies of red core root disease of the strawberry caused by *Phytophthora fragariae* Hickman

by

Isabel G. Montgomerie, B.Sc.


November 1951

# Studies of red core root disease of the strawberry caused by *Phytophthora fragariae* Hickman

CONTENTS

## INTRODUCTION.

Symptoms. Red core disease of the strawberry is one which attacks the young main and lateral roots of the plant. Main roots more than one year old are not infected. Infection usually takes place at the root tips and from there the disease progresses up the stele which turns a characteristic deep red in colour and this is followed by an externally visible brown rot of the outer tissues (Figs. 3 and 4). Laterals, more quickly killed than the main roots rot away leaving the root system much depleted of fibrous roots (compare Figs.1 and 2). The effect on the above-ground parts of the plant of this destruction of the roots, is the restriction of the normal development of the crown and foliage due to depleted water and nutrient supplies. Diseased plants make very little new growth and where the disease is unhindered by favourable weather conditions, e.g. heavy rainfalls, they eventually die. Although the disease is generally most active in the autumn, winter and early spring, it is in late spring and summer that the effects on the above-ground parts of the plant are most noticeable. At this time the developing foliage and fruit trusses are making increased demands on the root system which, if diseased, cannot supply sufficient nutrients or water for developing tissues. This results in dwarfed plants, and partial or complete wilting of the foliage, depending on the weather. Diseased plants usually occur in

patches in the field and these are most noticeable in the summer when the foliage of healthy plants is luxuriant.

Distribution. Owing to the rapidity with which red core may be transmitted from one plant to another in the field and the way in which incipient infection may go undetected in the roots of runners, it has spread in a comparatively short time to most of the strawberry growing areas in Scotland.

It was first recorded in Lanarkshire, Scotland, about 1920(40), and although for several years it was a serious menace to strawberry growers in this area only, it spread rapidly, no doubt by means of infected runners, until by 1941 it had been recorded in eighteen counties (30). By 1947 it was known that severe outbreaks had occurred in all the chief strawberry growing areas in Scotland. The first published account of an outbreak in England was by Wormald (44) in 1936 although it is probable that the disease had appeared in 1931 in Hampshire (19). From then on, outbreaks were noted in one locality after another until by 1950 it had been definitely identified in fourteen counties.

In 1935, Anderson (4) published an account of the disease in Illinois and Maryland in the U.S.A. Demaree and Darrow (15) in 1937 added to the list of states in which the disease was known to be present while Kadow (25), Valleau (38), Stoddard (34), Temple (36) and others later contributed information on the expanding list of areas infected in the U.S.A. At the

time of writing, red core has been recorded in twenty states.

In Canada, the first record was in British Columbia (12) in 1945 and by 1950 it had also been recorded in Nova Scotia (13) and New Brunswick (8).

The first account of the disease in New Zealand was in 1949 (7) when it was recorded in the three provinces of Aukland, Wellington and Canterbury.

In France where red core is known as apoplexy it has been reported from Plougastel, the Charentes and the vicinity of Paris (37).

Review of literature. The first published account of red core disease was by Wardlaw (40) in 1926, who referred to it as the"Lanarkshire disease". A description of the symptoms and development of the disease and of investigations into the cause of the condition was published by this author during 1926-1928 (40), (41),(42),(43). As a result of his observations Wardlaw came to the conclusion that there was no single organism responsible for the destruction of the root system but that the cause lay rather in poor cultivation. In 1928, O'Brien and McNaughton (29) advanced evidence which, in their opinion, indicated that the fundamental cause of the disease was endotrophic mycorrhiza in the roots and concluded therefore that cultural conditions alone were not responsible for the disease.

Alcock (1),(2), was the first to point out the significance of the oospores commonly found lying along the stele of infected roots and to describe the

oogonia, oospores and sporangia of the Phytophthora. Although attempts were made to isolate the fungus in pure culture, these were unsuccessful. Alcock also carried out inoculation experiments with healthy plants using chopped up diseased roots as inoculum and was successful in transmitting the disease for which she suggested the name'red core'.

In 1938, an account of the successful isolation in pure culture of the Phytophthora was published by Bain and Demaree in America (9). It was proved to be the causal organism of red core but was not identified. The following year Hickman (18) in England published an account of strawberry root rot in which he recorded the successful isolation of the Phytophthora causing red core. In 1940 the same author (19) published an account of the isolation and proof of pathogenicity and identified the fungus as Phytophthora fragariae n.sp. His pathogenicity tests showed that Fragaria was the only genus of those tested to be attacked by this Phytophthora. In 1945, Bain and Demaree (10) described further morphological and physiological studies of the fungus and confirmed Hickman's findings that Fragaria was the only genus to be attacked.

Long before the true cause of the disease had been clearly demonstrated, efforts had been made in Scotland to control its spread and to eradicate it from soil already infected (3). The first steps in this direction were concerned with various spray treatments, of which lime sulphur alone appeared to

exercise a temporary control, and soil sterilizers of which cresylic acid proved the most effective. Formaldehyde apparently produced no benefit. Together with these trials, additional drainage and deeper cultivation were also tried with similar lack of success in effecting a control of the disease. In 1933, it was decided that efforts should be concentrated on breeding new varieties resistant to the disease and to discontinue work on soil treatments (30).

A resistant seedling was found, the origin of which was unknown, but when crossed with other varieties it gave approximately 36% seedlings resistant to red core (30). This was the basis for the breeding programme carried out by Reid in Scotland and which has continued from 1933 to the present day.

Experiments had been started in America too to find resistant varieties (5). Only three were found to exhibit this characteristic - American Aberdeen, Mastodon and Redheart. The latter two were later found to be slightly infected. These three varieties were the basis of the breeding programme carried out in America by Darrow.

In 1938, Reid imported American Aberdeen to this country for incorporation in his breeding programme. After eight years of intensive field trials the first varieties resistant to red core were released to the trade in 1940. They carried the numbers of Auchincruive I,II,IV,V, and VI. American Aberdeen which had also undergone intensive trials in

this country was found to retain its resistance under Scottish conditions. In the same year, 1940, Hickman (19) had made observations on the reaction in the field of some commercial varieties after one year's trial. He found that Huxley, Tardive de Leopald, Madam Lefebvre, Western Queen and Sir Joseph Paxton were most susceptible, Royal Sovereign, The Duke, Bedford Champion, Aberdeen Standard, John Ruskin, Overtoun and Scarlet Queen were intermediate in reaction and Early Cambridge, Oberschlesien and Pillnitz were fairly resistant. Four of the Auchincruive varieties were found to be resistant the other one showing slight infection. After three seasons in commercial plantations all these Auchincruive varieties had been found infected although still showing a much higher degree of resistance than any of the other varieties then grown with the exception of American Aberdeen which had not then been found to be infected under Scottish conditions. Despite the increasing degree of infection noted in these resistant varieties, Reid (31) reported in 1944 that these varieties composed 75% of the total strawberry growing area in the Clyde Valley. In fact, wherever ground was heavily infected, they were the only varieties the growers could depend upon to produce a marketable sized crop and without them there is no doubt that a large section of the strawberry industry would have ceased to exist. The next resistant variety, bred by Reid, to be released to the trade was Auchincruive Climax which prior to the year of release, 1947, had been subjected to

intensive field trials for nine successive years without showing any signs of infection with the exception of one small plant which Reid had found in July of the previous year. This plant exhibited symptoms reminiscent of red core in one or two of the roots and when the roots were examined microscopically oospores were found in abundance. Although the oospores agreed in shape and general appearance with those of P. fragariae they were found to correspond in size to P. cryptogea rather than to P. fragariae. The measurements were:- Climax oospores ave. 23μ (21μ-31μ), P. cryptogea, ave. 22.8μ, P. fragariae, ave. 33μ(24μ-44μ). At a later date Climax plants were inoculated with a pure culture of P. cryptogea and no red core symptoms developed. As there was a good deal of doubt at this time that this was true red core infection and no further cases having been found prior to the date set for its release, Auchincruive Climax was released as a resistant variety. During the first season of growth under commercial conditions nine outbreaks of infection in this variety were reported from different localities all in Lanarkshire. These occurred on very heavily infected land and in most cases were associated with a badly drained part of the fields. Although the number of plants infected was relatively few, Reid reported that on individual plants the degree of infection was severe. Infected plants have since been found at Auchincruive, at two localities in West Lothian and have been reported from E. Sussex, Yorkshire and N. Ireland.

Darrow in America had received plants of

Auchincruive Climax for test and reported to Reid that under the conditions there this variety showed showed no resistance to the disease either in itself or as a parent. This information together with the fact that Anderson, also in America had in 1940 found Reid's earlier selections to exhibit the same resistance there, confirmed suspicions which had been held for some time by Reid and others that infection of hitherto completely resistant varieties must be due to the action of new races of the fungus.

Much the same story was unfolding in America where it was found that varieties resistant to red stele for a time in some parts of the country eventually became infected or did so in a different locality (24). Anderson and Colby (6) reported the slight infection of American Aberdeen in Illinois in 1942 although it was still showing complete resistance under Scottish conditions. Many authors contributed to the knowledge of resistant varieties in America among them Anderson (5), Darrow and Waldo (14), Temple (36), Jeffers and Darrow (23), and Colby and Boll (11). The methods of testing for resistance in use in the U.S.A. have been described by Jeffers (22), and Demaree and Jeffers (16). Waldo, Darrow, Jeffers, Demaree and Meader (39) summarise three types of testing - 1) naturally infected soil at Maryland Agricultural Experiment Station, 2) zoo-spore suspensions of the fungus for inoculating the soil of pots in which the test varieties are growing, resistant individuals then being planted in naturally

infected soil for fruiting trials, and 3) infected soil in the glasshouse. They found the third method to be the most economical and the most rapid.

Reasons for present investigation. As the entire position with regard to physiologic races in P. fragariae was in need of clarification the present investigation was undertaken at the suggestion of Dr. C. E. Foister of the Plant Pathology Laboratory, Dept. of Agriculture for Scotland, Edinburgh. As very little was known about the part played by the oospores in the initiation and perpetuation of the disease this was also considered worthy of further study. Two main lines of research were therefore indicated:-

I. Proof of the existence of physiologic races and the reaction of different varieties to infection.

II. The conditions governing the germination of the oospores.

- - - - - -

## I. THE OCCURRENCE OF PHYSIOLOGIC RACES AND THE DIFFERENTIAL RESPONSE OF VARIETIES TO INFECTION.

### ISOLATION OF THE PATHOGEN

In order to prove that physiologic races did exist it was essential to obtain, in pure culture, isolates of the fungus from different varieties of strawberry as well as from different localities. Bain and Demaree's (9) method of isolation was to select thick primary roots with healthy cortex and thoroughly wash in sterilised water. The cortex was then carefully removed under aseptic conditions and the upper two cms. of red stele cut in pieces 5mm. long. These pieces were then mounted on hardened drops of melted water agar and inverted over a Van Tiegham cell. When the fungus had been observed growing into the medium it was transferred to test tube slopes. Hickman (19) selected 1cm. lengths of infected root, surface sterilised them in .35% calcium hypochlorite solution afterwards washing them in several changes of sterilised water. Steles were then removed aseptically and placed on solidified agar. In a recent paper, Smith (33), using Hickman's method, reported the successful isolation of P. fragariae from infected plants in New Zealand.

A large number of isolations were made by the writer using both the above methods, but with no success. Other workers too have found the isolation of this Phytophthora to present some difficulty. As a result of failure to isolate the pathogen, all the

steps involved, from the selection of material for culturing to the plating out of pieces of infected roots on agar medium were critically examined. After many trials the following procedure was found to result in successful isolations:-

1) Immediately on lifting or not more than 12 hours afterwards, the roots, still attached to the plant, were thoroughly washed in running water until most of the soil particles were removed.

2) Thick main roots showing a small amount of rotting at the tip and if possible ones in which the red colouration had not extended to the rootstock were selected. After detaching from the plant the visibly rotted portion was cut off and the remainder placed in undiluted Chloros ( a proprietary product containing calcium hypochlorite) for three minutes. As a surface steriliser Chloros was found to be as effective as and easier to handle than either calcium hypochlorite or mercuric chloride.

3) The roots were then transferred to a Petri dish containing sterilised water where the cortex was removed aseptically and the upper 1cm. of reddened stele cut into two. The pieces of stele were placed on water-agar in a Petri dish and covered with a melted drop of the same medium as this was found to be the most effective method of reducing the activity of bacteria which were often present.

4) At a temperature of 15° - 20° C the charact - eristic hyphae of P. fragariae (Fig. 7) were observed four to five days after plating. As soon as the

growth of the Phytophthora was considered sufficiently vigorous the piece of stele and surrounding medium were transferred to a more favourable medium such as bean agar or quaker oat agar.

5) As has already been pointed out by Hickman the best times of the year for culturing are in the Autumn,Winter and early Spring when the fungus is growing actively in the roots.

It is not claimed that this method is superior to those already mentioned. Indeed on examination it will be seen to differ little from that described by Hickman. The success or failure of any method, in the writer's opinion, will depend primarily on the material used for culturing and secondly on the precautions taken to ensure that all operations, after surface sterilisation of the roots, are carried out as aseptically as possible.

The emphasis on using only fresh material may appear to be unnecessary but it was found that plants collected one day and placed in water over-night did not provide good material for culturing. The hyphae of P. fragariae may grow out from such material but they are usually swamped by faster growing fungi such as Pythium. Whether the increase of temperature from that of the soil to that of the laboratory allows the secondary invaders to lessen the distance between them and the advanced hyphae is not known. The change in the condition of the host may also have an effect on the relative positions in the diseased root of hyphae of the parasite and other organisms following

in its wake. It would seem that for successful isolation of this particular pathogen fewer liberties may be taken with the host-parasite relation than is possible with other disease producing Phytophthora spp. When it is impossible to collect and culture diseased material the same day it is advisable to pot up the diseased plants, keep them well watered in a cool place and attempt the isolation only when the roots are growing vigorously in their new medium.

Precautions to ensure aseptic conditions during culturing are particularly important with P. fragariae owing to its very slow rate of growth. When other fungi are present either on the root pieces or in the Petri dish their growth rate is usually so much faster than that of the Phytophthora that isolating the latter from them proves impossible in most cases.

The advantage in using water-agar for the primary isolation is two-fold :- 1) the hyphae of P. fragariae are distinguished more easily from those of other fungi than on media where growth is faster and 2) contaminating bacteria do not over-run the Phytophthora hyphae as quickly as on bean agar. The short period on water-agar appears to give the hyphae a vigorous start on bacterial contaminations and fewer cultures were lost when this medium was employed than when bean agar or oat agar were used in the preliminary stage of isolation.

Although it has been recommended that the upper 1cm. of discoloured stele be used, numerous isolations from all sections of the stele, above the

obviously rotted portion at the tip of the root, indicated that the most vigorous hyphae and fewest contaminations were not obtained from this region of the stele in every root. Comparisons between roots were made by cutting each main stele into 5mm. portions measured from the upper limit of red colouration. It was thus possible to gain information on the distribution and composition of the internal micro-flora of a number of infected roots. Clean cultures of P. fragariae were obtained from different portions of the stele in every root. In each stele there were usually three or four portions which gave such cultures and they were nearly always separated from each other by portions which produced secondary organisms only. It is probable that these organisms gain access to the main stele at various levels through lateral roots primarily invaded by P. fragariae. It is therefore impossible to forecast which parts of infected steles are likely to be entirely free from secondary organisms. However, in the writer's experience it was found that the portion in most roots to yield a high proportion of cultures free from contaminations was the upper 1 cm. of reddened stele.

Hickman (19) and Anderson (5) have observed that attempts to isolate the fungus from above the red colouration in the stele were negative. On two occasions the writer obtained vigorous hyphae of the Phytophthora from the 1 cm. portion of the stele immediately above the upper limit of the red colouration. In the first instance, when it was being

determined which part of infected steles gave the best results for culturing, two roots were used in both of which the red colouration ceased some distance from the rootstock. The portion of the stele 1 cm. in length immediately above the limit of red colour was cut out in both cases and then halved. In the first root, vigorous hyphae were obtained from both portions, in the second, hyphae were obtained only from that portion which represented the 5 mm. immediately above the limit of the red colour. The pieces of stele were observed five days after plating and in all instances, even in the portion which did not produce hyphae, the steles were now coloured red. This was repeated with three similar roots, two of which gave negative results and the third of which produced hyphae from the 5 mm. portion immediately above the limit of red colouration. In all instances however it was noted as before that although when plated, all these particular sections of the stele were white, when they were examined five days later they were all, without exception, red. These results would appear to indicate that the colour change is not an effect in advance of of the mycelium or one which occurs simultaneously with the penetration of the hyphae into the vascular tissues but is the result of the metabolism of the hyphae within the cells and occurs some time after penetration has taken place.

As hyphal tip isolations were desired, mycelium of the various isolates was transferred to bean agar on which the hyphal tips were cut off by means of a

hollow objective. The hyphal tips were then transferred to oat agar on which growth was rapid. Ten of the isolates obtained in culture were retained for study, and Table 1 gives details of their origin and where infection is believed to have been contracted from the soil, the cropping history of the field in which the strawberries were grown is also given.

Table 1. Origin of isolates

| Isolate | Variety | Locality | History of Field |
|---|---|---|---|
| 70 | unknown | unknown | --- |
| 71 | Auch. 2 | Auchincruive, Ayrshire. | seedling varieties, Huxley, L.R. 19, ect., grown every year |
| 72 | Auch. 5 | ditto | ditto |
| 74 | Auch. Climax | Carluke, Lanarkshire. | --- |
| 78 | Royal Sovereign | Edinburgh | in grass for 15 years |
| 81 | Huxley | Lothians | --- |
| 89 | Auch. Climax | Kirkfieldbank, Lanarkshire. | --- |
| 91 | Auch. 1 | East Craigs, Edinburgh. | --- |
| 92 | Auch. 2 | ditto | --- |
| 93 | Auch. Climax | West Lothian | --- |

Growth in pure culture. Of the media tested, and these included Quaker oat agar, bean agar (made from broad beans ), lima bean agar, potato dextrose agar, hemp seed agar, malt agar and water agar, Quaker oat was found to be the best for maintaining cultures. The growth rate though initially slow was eventually faster than on any of the others. No sporangia were

observed on any of the above media and oospores were found only in deep oat agar tubes after two to three weeks or occasionally on month-old slopes. No growth was made when water-agar discs containing mycelium were transferred to malt agar but some growth did take place when discs of bean agar containing the fungus were placed on this medium. The type of growth (Fig.8) which took place was abnormal and was presumably due to the food substances still derived from the bean agar disc.

COMPARISON OF ISOLATES IN PURE CULTURE

In order to find out if the isolates differed in the size of spores they produced, and by a comparison with their pathogenicity, if physiologic races could be distinguished by differences in size of spores, random samples of twenty-five of each type of spore i.e. sporangia, zoospores at rest, and oospores were measured. The data were treated statistically and the means for each isolate tested for significant differences.

Production of sporangia and zoospores. Hickman (19) observed that in cultures of the fungus on hemp seeds in Petri's solution, the production of sporangia and zoospores was abundant while Bain and Demaree (9) found that water from melted snow gave excellent results. The preparations used by the latter for an investigation of the temperature relations of sporangia and zoospores were thin blocks of lima bean agar containing mycelium irrigated with a shallow layer of tap water. The latter method was tested whilst

investigating suitable means of preparing sporangia and zoospores for measurement but it was found that under the conditions used by the writer only a small quantity of sporangia were produced. Sporangia were formed in small quantities also when cultures of the fungus on hemp seed, and on an agar medium prepared with hemp seeds, were placed in Petri's solution.

As neither of these methods appeared to be suitable, different media and liquids were tested. Discs were cut out from near the margin of actively growing colonies as it had been found that old cultures were unsuitable for sporangial production. They were then placed in a shallow layer of the liquid to be tested either in a Petri dish or more usually on glass slides. The temperature used was 10°-20° C. A summary of these tests is given in Table 2. The observations given are not accurate assessments of the effect of the different treatments on sporangial production as it was not found possible to make precise counts of sporangia on those discs which gave large numbers. It was therefore possible to make only rough comparisons between two or more treatments and to discard those which were obviously not producing sporangia in quantity while trying to improve on those which were. The soil which was used was John Innes potting compost.

Since cultures of the fungus on bean agar discs in soil leachate gave a satisfactory production of both sporangia and zoospores this was the method used in obtaining these spores for measurement.

Table 2. Production of sporangia

| Media and liquids compared | Observations |
|---|---|
| bean, Quaker oat, hemp seed ) agar + tap water containing a suspension of soil particles | sporangia formed on all media, more abundant on bean and oat than on hemp seed agar |
| bean, Quaker oat, hemp seed ) agar + Petri's solution | sporangia formed on all media abundant on oat agar few on hemp seed and bean agar |
| bean agar + (a) peat leachate (b) soil leachate | sporangia more in (b) than in (a) |
| bean agar + (a) soil leachate prepared by collecting drainage water through filter paper (b) soil leachate prepared by allowing soil to stand for 48 hours in water and filtering | sporangial production slightly greater in (b) than in (a) |
| bean agar + soil leachate (a) untreated (b) placed in steamer for 15 minutes (c) steamer for 30 mins. (d) ,, ,, 1 hour (e) ,, ,, 1 ,, on three consecutive days (f) autoclaved for 20 minutes at 20lbs. pressure | very little difference between (a) and (b) and between (c),(d),(e) and (f) but the number of sporangia produced in (a) and (b) was greater than in any of the others |

Sporangia. For the comparison of sporangium and zoospore sizes, standard discs ( 2mm. in diameter and of uniform depth ) were cut out from actively growing colonies of the isolates on bean agar, placed on glass slides and the same volume of soil leachate added to

each disc. The slides were then placed in a damp chamber at a temperature of 15-20°C, the soil leachate being changed daily. When sufficient sporangia had formed, cover-slips were placed over the discs and measurements made. The mean length and breadth of the sporangia are given in Table 3.

Table 3. Mean length and breadth of sporangia

| Isolate | Source | Length | | Breadth | |
|---|---|---|---|---|---|
| | | micr. divs. | μ | micr. divs. | μ |
| 70 | unknown | 140 | 39.9 | 86 | 24.5 |
| 71 | Auchincruive 2 | 133 | 37.9 | 87 | 24.8 |
| 72 | Auchincruive 5 | 135 | 38.5 | 87 | 24.8 |
| 74 | Auchincruive Climax | 137 | 39.0 | 87 | 24.8 |
| 78 | Royal Sovereign | 124 | 35.3 | 85 | 24.2 |
| 81 | Huxley | 126 | 35.9 | 87 | 24.8 |
| 89 | Auchincruive Climax | 120 | 34.2 | 87 | 24.8 |
| 91 | Auchincruive 1 | 116 | 33.1 | 73 | 20.8 |
| 92 | Auchincruive 2 | 126 | 35.9 | 82 | 23.4 |
| 93 | Auchincruive Climax | 130 | 37.0 | 86 | 24.5 |
| S. E. of difference between means | | ± 7.78 | | ± 4.07 | |
| Difference required for significance at .1% level | | 25.6 | | 13.4 | |

As the statistical analyses were carried out using micrometer divisions as the unit of measurement, the means in this unit must be used in testing for significance. An examination of the data in Table 3 shows that the differences in length and breadth of

sporangia are not great enough to be significant at the level of probability chosen, the difference (14) between the breadth of sporangia in isolate 91 and isolates 71,72,74,81,and 89 being only just greater than the calculated value (13.4). Where more than one isolate was found by pathogenicity tests (Table 8) to be a member of the same physiologic race, the mean dimensions were calculated for the group as a whole and compared with the means of the other races. There was no indication here either that significant differences in sporangium size occurred. Compare the means of isolate 81 (race 1) with isolates 71,72,78, 91 (race 2) and with isolate 74 (race 3).

A comparison of the dimensions of the sporangia shown above with those recorded for P. fragariae by Hickman (19) shows that both length and breadth although falling within the range given (32-90×22-52)μ are smaller than the average recorded by this author (60 ×38)μ. These measurements were however made from sporangia growing on infected roots and although the number of spores measured is not stated the difference in average size may not be due to the smaller sample of spores measured by the writer but to the fact that they were grown in pure culture on small amounts of medium. This does not necessarily invalidate the comparison as presumably if there had been any tendency for any of the isolates to produce bigger sporangia these would still have been produced although in smaller numbers.

Zoospores. Zoospores were obtained from mature

sporangia at intervals usually after the addition of fresh soil leachate or in the morning as a result of the lower temperature prevailing over-night. Slides were selected on which a good number of zoospores were actively swimming. After a time they came to rest assuming a spherical shape. The diameters of the resting zoospores were then measured. Table 4 gives the mean diameters in micrometer divisions (which have to be used in tests for significance) and in micra.

Table 4.
Mean diameter of zoospores

| Isolate | Source | Micr. divs. | μ |
|---|---|---|---|
| 70 | unknown | 47 | 13.4 |
| 71 | Auchincruive 2 | 42 | 11.9 |
| 72 | Auchincruive 5 | 47 | 13.4 |
| 74 | Auchincruive Climax | 40 | 11.4 |
| 78 | Royal Sovereign | 45 | 12.8 |
| 81 | Huxley | 41 | 11.7 |
| 89 | Auchincruive Climax | 42 | 11.9 |
| 91 | Auchincruive C 1 | 43 | 12.2 |
| 92 | Auchincruive 2 | 42 | 11.9 |
| 93 | Auchincruive Climax | 45 | 12.8 |
| S.E. of difference between means | | ±1.45 | |
| Difference required for significance at .1% level | | ±4.9 | |

The diameter of zoospores in isolates 70 and 72 were just significantly higher than those of the rest except 78,91 and 93. Isolates 78 and 93 were also significantly higher than 74. Only one of the isolates (78) of race 2 could be distinguished by size from those of another race (isolate 74 of race 3) but when the average diameter for all the isolates of race 2 was calculated, no significant difference was found between it and the mean diameters

of the other two races. Thus, although there are significant differences between isolates when the diameter of the zoospores at rest are compared, there is no correlation between these differences and the physiologic races. Also, since the difference in size between the largest and smallest mean amounts to only two micra it is doubtful if such statistically significant differences as do occur have any meaning for the mycologist.

The size of the zoospores agrees more closely with that recorded by Hickman (19) - 12μ, than that found by Bain and Demaree (10) - 10μ. The range in size of zoospores over all the isolates was relatively wide extending from one at 8.8μ to one at 17.9μ.

Oospores. In order to obtain oospores in pure culture for measurement, deep oat agar tubes were inoculated with the isolates and left for three months. At the end of that time oospores although never abundant were fairly numerous in some tubes whilst in others they were few in number and hard to find. They were mounted in lactophenol and the diameters measured. Whilst doing this, observations were made on the type of antheridium present where the structure and position could be clearly seen. Owing to the closely adhering medium and the deeply stained oogonial envelope this was possible in only fifteen cases. In nine of these the antheridia were paragynous and in six amphigynous. These observations although small in number tend to support the conclusion eventually arrived at by Alcock, Howells and Foister (3) that the two types

of antheridia were formed in about equal proportions.

Table 5 gives the mean diameter of the oospores and in this case the analysis was carried out using the micron as the unit of measurement so that the standard error applies to the means given.

Table 5.
Mean diameter of oospores

| Isolate | Source | $\mu$ |
|---|---|---|
| 70 | unknown | 29.0 |
| 71 | Auchincruive 2 | 28.9 |
| 72 | Auchincruive 5 | 32.6 |
| 74 | Auchincruive Climax | 32.0 |
| 78 | Royal Sovereign | 30.8 |
| 81 | Huxley | 31.3 |
| 89 | Auchincruive Climax | 28.8 |
| 91 | Auchincruive 1 | 30.0 |
| 92 | Auchincruive 2 | 31.3 |
| 93 | Auchincruive Climax | 29.4 |
| S.E. of difference between means | | ±1.19 |
| Difference required for significance at .1% level | | 4.0 |

As will be seen from Table 5, there was no difference in size of any two isolates sufficiently great to be considered significant at the level of probability chosen, the greatest difference (3.8) being that between isolate 89 which had the smallest diameter and isolate 72 with the largest.

When compared with the measurements of oospores given by Hickman (19) all the isolates fall within the range given (24-44)$\mu$ but are slightly smaller than the average (33$\mu$).

It was found that considerable variation in size occurred in all the spore types despite the standardised conditions. As will be seen from Table 6 the greater the magnitude of the measurements taken, the greater the variation.

Table 6. Magnitude of variation in spore size

| Dimensions | | Mean variation in size within isolates (μ). |
|---|---|---|
| Sporangium | length | 29.3 |
| | breadth | 14.7 |
| Oospore | diameter | 15.8 |
| Zoospore | diameter | 5.5 |

The above results indicate that there are probably no significant differences in spore size between the physiologic races of P. fragariae.

Temperature relations. In order to discover if the isolates reacted similarly to different temperatures, discs (2 mm. in diameter) containing mycelium were transferred to plates of lima bean agar. Three plates were inoculated with each isolate and the average diameter of the resulting colonies was calculated. The diameter of each colony was measured in two directions at right angles and the mean taken.

The minimum temperature. One series of plates was placed at 1.5°C and after 10 days no growth had been made by any of the isolates. That none of them had been killed by exposure to this temperature was demonstrated by transferring the plates to 20°C where growth was normal. Another series of plates was placed at 3°C and after ten days all the isolates had made some growth (Table 7). This temperature would therefore appear to be near the minimum for all isolates. The temperature recorded as the minimum for P. fragariae by both Hickman (19) and Bain and Demaree (10) was 14°C.

The optimum temperature. Three series of plates were set up at 18°C, 20°C, and 22°C. The amount of growth recorded at these temperatures is shown in Table 7.

Table 7. Diameter of colonies on lima bean agar (after 14 days)

| Isolate | Source | Diameter of colony (mm) | | | |
|---|---|---|---|---|---|
| | | 3°C (after 10 days) | 18°C | 20°C | 22°C |
| 70 | unknown | 6 | 31 | 58 | 53 |
| 71 | Auchincruive 2 | 6 | 40 | 50 | 52 |
| 72 | Auchincruive 5 | 4 | 36 | 54 | 51 |
| 74 | Auchincruive Climax | 4 | 38 | 68 | 58 |
| 78 | Royal Sovereign | 4 | 36 | 56 | 59 |
| 81 | Huxley | 5 | 26 | 54 | 69 |
| 89 | Auchincruive Climax | 4 | 40 | 61 | 48 |
| 91 | Auchincruive 1 | 6 | 36 | 66 | 68 |
| 92 | Auchincruive 2 | 4 | 43 | 52 | 64 |
| 93 | Auchincruive Climax | 4 | 26 | 61 | 59 |

All the isolates made more growth at 20°C than at 18°C, the amount of growth at both temperatures varying considerably between isolates. Five of the isolates (71,78,81,91 and 92) made more growth at 22°C than at 20°C, the remaining isolates making less growth. It seemed therefore that the optimum temperature was not the same for all isolates, of some being near 20°C and of others nearer 22°C. There was no evidence that isolates of one physiologic race had the same optimum temperature, as three of the isolates

(71,78,and91) of race 2 made more growth at 22°C while the fourth isolate (72) made less growth at this temperature than at 20°C. The optimum temperature of P. fragariae recorded by Hickman(19) was 20°C and by Bain and Demaree(10) was between 18°C and 22°C.

The maximum temperature. None of the isolates grew at 30°C and after ten days no growth took place when they were transferred to 20°C. At 28°C, no growth had been made by any of the isolates after fourteen days. This temperature was not lethal as growth took place when the plates were transferred to 20°C.

PATHOGENICITY OF ISOLATES

The differentiation of physiologic races necessitated the use of a panel of indicator varieties differing in their reaction to infection by red core. The following five varieties, Huxley Giant, Oberschlesien, Auchincruive 6, Auchincruive Climax, and Auchincruive 11, were selected because they appeared to provide the widest range of reaction to the disease as far as could be judged from their performance in naturally infected fields. As a certain amount of confusion exists in the use of such terms as 'immune' and 'resistant', it may be adviseable here to describe the field reaction to disease of the above varieties instead of attempting to place them in any specific category.

Huxley Giant: severely attacked, most roots becoming infected, and where environmental conditions are favourable for the pathogen, plants may be killed in one or two seasons.

Oberschlesien: becomes infected but many reports indicate that it is able to survive for longer periods than Huxley on infected land.

Auchincruive 6: one of the earlier varieties introduced by Reid. It was bred for resistance and did not become infected under test. Some time after its release plants in a number of localities became infected. According to some reports even when this variety is infected it still survives longer than Huxley.

Auchincruive Climax: also raised by Reid and found by him to remain uninfected after many years of test. After its release in 1947, a number of plants became infected in some places. Although individual plants are sometimes found to be very heavily infected, it is too early to say yet how this variety when infected will compare with Huxley.

Auchincruive 11: a recent selection by Reid which is not to be released for reasons other than red core resistance. It had not been found to be infected either under natural or artificial conditions of infection.

It was at first planned to root runners from a selected number of plants of each variety into sterilised soil and to use the young plants for testing. Owing however to a severe infestation of Tarsonemid mite the number of young plants was considerably reduced and although this pest was eventually eradicated by fumigating with methyl bromide, a large number of replacements had to be made by collecting

young plants from the field. These were all obtained from certified stock growing on land which had no history of red core. As it was hoped to inoculate all plants at approximately the same stage of development, all roots were cut off leaving only very young primary roots on each plant. The plants were kept well watered until they had re-established themselves. When the pots were filled with actively growing root systems, the plants were inoculated.

Owing to space limitation, only six isolates were tested in this experiment. They were:-

isolate 81 ex Huxley
.. 91 .. Auchincruive 1
.. 72 .. Auchincruive 5
.. 78 .. Royal Sovereign
.. 71 .. Auchincruive 2
.. 74 .. Auchincruive Climax

These isolates were multiplied on bean agar in Petri dishes. The method of inoculation used was similar to that described by Hickman and English(35). When the colonies were two to three weeks old, discs 1.1 cm. in diameter were cut out from all but the oldest portions of the colony with a cork borer. Although an attempt had been made when pouring the plates to ensure the even distribution of bean sediment throughout the plate, and also to have a uniform thickness of agar, differences in both these factors were noted when the discs were cut. All the discs for each isolate were therefore bulked in a large Petri dish containing tap water and well mixed. When the plants were inoculated ten discs were selected at random for each plant and in this way differences in inoculum

were distributed throughout the treatments. Ten plants of each variety were inoculated with each of the six isolates making thirty treatments with ten replicates.

As it was necessary to have the inoculum distributed as equally as possible around the plant and also to have the distribution of inoculum as uniform as possible from plant to plant the following system of placing the inoculum was carried out. Two rings of five vertical slits were made, with a spatula, in the soil at approximately equal distances from each other and from the crown of the plant. In the inner ring of slits were dropped five discs just below the surface of the soil near the crown of the plant and into the outer one five similar discs were placed so that they were about one inch below the surface of the soil, near the edge of the pot. They were then lightly covered with soil. In preliminary inoculations the vertical arrangement of discs was found to give a greater amount of infection than discs placed horizontally in the soil. Not enough trials were carried out to ensure that this result did not occur by chance but it could be explained by the fact that particles would tend to lodge more readily on a horizontal surface than on a vertical one. This might cause the Phytophthora on the disc to be over-grown by faster growing fungi such as Penicillium before large numbers of sporangia could be produced. There is also the possibility that the oxygen available for sporangial production would be reduced as a result of this clogging of the surface of the disc in the

horizontal arrangement.

Before inoculation the soil in all pots was saturated with water, thereafter they were watered twice daily. The experiment was terminated after four weeks. The temperature of the soil in the pots though frequently below 20°C rarely rose above this temperature.

Lay-out of the experiment. This was a randomised block arrangement in which the thirty treatments were randomised in each of the ten blocks. The block of three hundred plants on the glasshouse bench was surrounded by a row of un-inoculated plants in order to equalise the environment in the outer and inner rows.

Observations. At the end of four weeks, the plants were removed from the pots, a block of thirty at a time, and after washing the root systems free from soil, the following observations were made on the reaction of each plant to inoculation:-

Reaction; the presence or absence of red core symptoms on the roots (where no red cores were found on the main roots, the laterals were carefully examined and where red cores were found on them alone the reaction was still considered to be positive).

Infection; the proportion of main roots showing symptoms of red core (i.e. the number of diseased roots divided by the total number of roots).

Penetration; the average length of red core per root calculated by dividing the total length of red core by the number of diseased roots.

Prior to the observations made in this experiment all the cases of red core examined by the writer showed the red colouration of the stele proceeding from the root tip (Figs. 3 and 4). This was also observed by Hickman (19). In many of the roots examined in this experiment however the red colouration did not originate in the root tip of the main root and when these roots were examined microscopically oospores were seen in the reddened portions of the stele but neither oospores nor sporangia were observed at the tip which showed no externally visible sign of rot (Figs. 5 and 6). In all the roots examined there was always an infected lateral at some point on the reddened portion but whether infection had proceeded into the main stele from them or directly through the cortex was not determined.

Results. Reaction. The number of plants which showed symptoms of red core after inoculation is shown in Table 8.

A study of the reactions of the five varieties indicates the presence of three physiologic races. Isolate 81 ex Huxley is apparently one race (race 1) identified by its non-pathogenicity to Auchincruive 6, Auchincruive Climax and Auchincruive 11. Isolates 791 ex Auchincruive 1, 72 ex Auchincruive 5, 78 ex Royal Sovereign and 71 ex Auchincruive 2 represent another race (race 2) non-pathogenic to the varieties Auchincruive Climax and Auchincruive 11. Isolate 74 ex Auchincruive Climax is distinct as the third race (race 3) which is pathogenic to all the varieties

tested.

Table 8. Reaction (no. of plants infected out of 10)

| Isolate | Source | Indicator varieties | | | | |
|---|---|---|---|---|---|---|
| | | Huxley | Ober-schlesien | Auch.6 | Auch. Climax | Auch.11 |
| 81 | Huxley | 9 | 5 | 0 | 0 | 0 |
| 78 | Royal Sov. | 10 | 8 | 3 | 0 | 0 |
| 91 | Auch.1 | 9 | 8 | 10 | 0 | 0 |
| 72 | Auch.5 | 10 | 10 | 10 | 0 | 0 |
| 71 | Auch.2 | 10 | 8 | 9 | 0 | 0 |
| 74 | Auch. | 10 | 9 | 9 | 10 | 1 |

The low number of plants of Auchincruive 6 infected by isolate 78 ex Royal Sovereign and of Auchincruive 11 infected by 74 ex Auchincruive Climax will be noticed in Table 8. Considering the number of plants infected in the other treatments it seems unlikely that such low figures could be due to disease escape. This possibility however cannot be over-looked and further tests are in progress to obtain more information on these two treatments. It will be seen also that with the exception of Auchincruive 6, the Auchincruive varieties were not infected by the isolates derived from Huxley and Royal Sovereign but were infected only by the isolates obtained from Auchincruive varieties.

Infection. The percentage of roots found to be infected is shown in Table 9. A statistical analysis was carried out omitting the varieties Auchincruive

Climax and Auchincruive 11 owing to their non-infection by all isolates except 74, the data being transformed using the angular transformation (Fisher and Yates(36) Table xii). The transformed values are given in brackets in Table 9 as they must be used in tests of significance.

Table 9. Infection(percentage of main roots infected)

| Isolates | Indicator Varieties | | | Means | |
|---|---|---|---|---|---|
| | Huxley | Ober-schlesien | Auch.6 | | |
| 81 ex Huxley | 64 (53.1) | 11 (19.5) | 0 (0.0) | 17 (24.2) | |
| 78 ex Royal S. | 77 (61.2) | 23 (28.5) | 2 (7.2) | 29 (32.3) | S.E. of difference of means ± 5.2 |
| 91 ex Auch. 1 | 67 (54.7) | 8 (15.9) | 64 (52.8) | 43 (41.1) | |
| 72 ex Auch. 5 | 64 (53.2) | 27 (31.2) | 65 (53.7) | 52 (46.0) | |
| 71 ex Auch. 2 | 72 (57.8) | 14 (21.7) | 48 (43.7) | 43 (41.1) | Auch. Auch. Climax 11 |
| 74 ex A. Climax | 67 (54.8) | 13 (21.5) | 58 (49.6) | 45 (42) | 77 1 (61.3)(3.1) |
| Means | 68 (55.8) | 15 (23.0) | 32 (34.5) | | |

S.E. of difference of means ± 3.7

S.E. of difference of two values in centre of table ± 9.0

Differences between varieties were found to be highly significant, the proportion of roots infected in Oberschlesien being lower than the proportion infected in all the other varieties except Auchincruive

11. This was not due to the production of more roots by Oberschlesien as the average number of roots formed per plant differed little from the number formed by Huxley and Auchincruive 6 and was less than the number formed by Auchincruive Climax and Auchincruive 11. There was no difference between Huxley, Auchincruive 6 (omitting isolates 81 and 78) and Auchincruive Climax (considering isolate 74 alone).

The differences between isolates was also found to be highly significant but this was due to the low proportion of roots infected in Auchincruive 6 by isolates 81 and 78. When the latter were omitted there was no difference in the proportion of roots infected by the different isolates or races.

There was no difference between the proportions infected by different isolates on the same variety except in the case of Auchincruive 6 where both isolates 81 and 78 caused a significantly lower amount of infection than the rest.

The very low proportions recorded for Auchincruive 6 infected by isolate 78 and Auchincruive 11 infected by isolate 74 are to some extent due to the small numbers of plants infected (Table 8), but the percentage of diseased roots in each of these plants was also low. The figures recorded were, 29, 13, and 10 in Auchincruive 6 and 26 in Auchincruive 11.

The results in Table 9 indicate that if either races or isolates differ in aggressiveness it is not evident when the above assessment of infection is used.

Penetration. The average penetration per main root based on the length of red core in cms. is shown in Table 10. In those treatments where infection had occurred in some of the plants but not in others, it was assumed for the purposes of the statistical analysis that they were due to disease escape. Missing values were therefore calculated in order to obtain an unbiased estimate of error. The numbers in brackets in Table 10 are the numbers on which means are based and are the number of plants in each treatment in which infected main roots were found. In comparing the means for significant differences the formula, S.E.$(m_1 - m_2)$ $= \pm S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$ (where S = standard deviation, S.E. = standard error, $m_1$ and $m_2$ = means of $n_1$ and $n_2$ measurements respectively) was used.

Table 10. Penetration
(average length of red core in cms.)

| Indicator Varieties | Isolates | | | | | | Means |
|---|---|---|---|---|---|---|---|
| | 81ex Huxley | 78 ex Royal Sov. | 91 ex Auch. 1 | 72 ex Auch. 5 | 71 ex Auch. 2 | 74 ex Climax | |
| Huxley | 9.1 (9) | 7.9 (10) | 7.5 (9) | 6.6 (10) | 7.2 (10) | 7.2 (10) | 7.6 |
| Ober-schlesien | 5.2 (5) | 4.7 (8) | 4.8 (5) | 5.2 (7) | 4.7 (7) | 5.8 (7) | 5.1 |
| Auch. 6 | 0 (10) | 2.1 (3) | 6.6 (10) | 6.0 (9) | 6.8 (9) | 6.5 (8) | 4.7 |
| Means | 4.8 | 6.3 | 5.9 | 4.9 | 6.2 | 6.5 | |

Standard deviation = ± 2.31

| | |
|---|---|
| Auch. Climax | 8.8 (10) |
| Auch. 11 | 4.9 (1) |

When the penetration recorded in each variety was compared statistically it was found that the penetration in Oberschlesien was significantly lower than that in Huxley, Auchincruive 6 (when isolates 81 and 78 were omitted) and Auchincruive Climax (when isolate 74 alone was considered). There was no significant difference between Huxley and Auchincruive Climax which were only just significantly greater than Auchincruive 6 at the 5% point.

When the effect of the isolates on Huxley and Oberschlesien alone was considered it was found that neither they nor the physiologic races differed in the amount of penetration recorded. Similarly, when the three varieties were considered, if isolates 81 and 78 be omitted, they did not differ. There is therefore no evidence that there is any difference in the agressiveness of physiologic races when the average length of root penetrated by them is used as the basis of assessment.

The penetration of isolate 78 in Auchincruive 6 was the lowest recorded whilst that of 74 in Auchincruive 11 was comparable with the values recorded in Oberschlesien.

## DISCUSSION

The comparison of the reaction of five varieties of strawberry to inoculation with P. fragariae under standardised conditions has shown that, apart from their differential response to the physiologic races present, they differ in the proportion of roots which become infected and in the extent to which the disease

progresses into the tissues. It has been demonstrated (Tables 9 and 10) that in the variety Oberschlesien, a proportionately smaller number of roots becomes infected and the extent to which the pathogen penetrates these roots is less than in Huxley. This would account for the performance of the former variety in the field, where it survives for longer periods than Huxley on infected land. Auchincruive Climax, on the other hand, was infected as severely as Huxley when it was inoculated with a race pathogenic to it. The variety Auchincruive 6, although not differing from Huxley in its reaction to four of the isolates, when infected by isolate 78 from Royal Sovereign, exhibited the smallest amount of penetration recorded and a lower proportion of infected roots than Oberschlesien. In the only plant of Auchincruive 11 to be infected, the penetration was similar to that recorded for Oberschlesien but the proportion of infected roots was much lower.

It would appear therefore that in the varieties tested there are at least two factors involved in the resistance of the host to the parasite. There is, firstly, the factor present in the three Auchincruive varieties which is evidently effective in resisting infection by specific races. Secondly, there is the factor present in Oberschlesien which is not effective in preventing infection but slows down the rate of spread of the parasite within the tissues. Whether the small amount of penetration recorded in Auchincruive 6 infected with isolate 78 ex Royal

Sovereign and in Auchincruive 11 infected with isolate 74 ex Auchincruive Climax resulted from the operation of the first factor, the second, or both, is not clear. It is possible that the defence reaction or whatever mechanism the first factor controls, varies in the extent to which it is effective and that the examples just given are instances of a reaction, intermediate in character, between one where infection is prevented and one where a specific race completely overcomes the defence mechanism and a susceptible reaction results. The reason for the small proportion of infected roots in Oberschlesien, in Auchincruive 6 infected with isolate 78 ex Royal Sovereign, and in Auchincruive 11 infected with isolate 74 ex Auchincruive Climax is not understood. It is possible that these results were due to disease escape which did not occur to the same extent in Huxley because of the secondary inoculum available from infected roots and it may be significant that all instances of low proportions of infected roots were associated with lower amounts of penetration than were recorded for Huxley. If it is assumed that sufficient inoculum was present to give all roots an equal chance of becoming infected, the above results would appear to suggest that roots of the same plant do not necessarily react in the same way to inoculation; that some roots when in contact with the disease organism become infected while others do not. It should be possible to gain more information on this point by more precise methods than have been possible

here. Since it has been discovered by the writer that detached roots, when inoculated with a zoospore suspension of P.fragariae in a Petri dish, give the same disease reaction as living plants inoculated in the manner described in the foregoing pages, it seems likely that this technique will provide a way in which to study the defence reactions of different varieties.

Until more is known about the mechanism of resistance to red core, it is not possible to classify varieties precisely according to their type of reaction to the disease. The "susceptible" varieties offer no difficulty but when the terms "resistant" and "immune" are used it might reduce the confusion existing in the literature if these words , when used, were qualified by a few remarks on their real meaning with regard to the variety they are used to describe. It would also be helpful to workers in other localities to be told what methods of test had been used in arriving at such classifications. The problem would still remain, however, of describing accurately but briefly for the benefit of growers the probable reaction to disease in the field of new varieties.

It has been described how resistant varieties of strawberry may be grown for a number of years on heavily infected land without contracting the disease and yet a short time after coming into commercial cultivation they become infected. There is no evidence that this is due to the action of newly evolved physiologic races but may be the result of the large multiplication of plants which takes place increasing

the probability of some of the units of a variety coming into contact with a race which is pathogenic to it.

During the course of this investigation, Scott, Jeffers, Darrow and Ink (37) in America have published an account of work which indicated the presence of at least two physiologic races designated by them A and S. These authors used infected soil from different localities as the source of inoculum and found that American Aberdeen and other American varieties which had been bred for resistance were susceptible to race A but resistant [some plants were slightly infected] to race S whereas varieties derived from crosses with resistant Scottish selections were susceptible to race S but not to race A [again, some plants were slightly infected]. In a recent paper Hickman and English (38) have demonstrated the presence of at least three physiologic races in Great Britain. The indicator varieties they used were Huxley, Perle de Prague and Auchincruive Climax and infection was assessed by calculating the length of red core as a percentage of the overall root length. In this way isolates were classified as being highly pathogenic, slightly pathogenic and non-pathogenic. Race 1 was differentiated by its pathogenicity to Huxley, slight pathogenicity to Perle de Prague and non-pathogenicity to Auchincruive Climax. Race 2 was found to be highly pathogenic to Huxley and Perle de Prague and non-pathogenic to Auchincruive Climax. Race 3 was

pathogenic to Huxley, Perle de Prague and Auchincruive Climax. It is probable that race 1 and race 3 found by Hickman and English are identical to race 1 and race 3 described by the writer. Since the other indicator variety was different it is not possible to judge whether race 2 is the same also. It will be noted that the above authors consider the difference between the slightly pathogenic category and highly pathogenic one to be sufficient for the differentiation of race 1 and race 2. In the writer's experiment only pathogenic and non-pathogenic categories were used in the differentiation of races. Should further work prove that different races may be distinguished by such differences in percentage of infection as Hickman and English have used, it may be that in the writer's material there are four races instead of three, isolate 78 ex Royal Sovereign being the additional one distinguished from the other isolates of race 2 by its slight pathogenicity to Auchincruive 6.

The origin of these physiologic races is not known. It is possible that with the multinucleate coenocytic mycelium typical of P.fragariae and other spp. of this genus, heterokaryosis may exist although there is no evidence,as far as the writer is aware, that such a condition occurs in any member of this genus. If it should prove to be the case however numerous opportunities for the segregation of different races must occur. There is also the possibility that the races arise as a result of spontaneous mutations. With regard to both these possibilities, it may be

significant to note that so far P. fragariae has not been observed to dissociate into different forms in the laboratory under pure culture conditions. This phenomenon has been observed to occur in P. parasitica var. rhei by Leonian (39),(41),(42).

Although it has been shown, by hyphal tip transfers, that P. fragariae is homothallic, races may arise by hybridisation through fertilisation of oogonia of one race by antheridia of another.

The effect of different varieties on the parasitic range of a race has yet to be investigated and it may be found that new forms arise through a process of adaptation. This would mean that the resistant host was a necessary factor in the evolution of new races and not simply a filter by which different races already present could be identified. Some evidence exists that a race pathogenic to a previously resistant variety may arise through no intervention of resistant varieties. In Table 8 it will be seen that isolate 78 obtained from the susceptible variety Royal Sovereign was pathogenic to the resistant variety Auchincruive 6. The source of infection of these Royal Sovereign plants was investigated and it seemed fairly certain that no contact with resistant varieties could have occurred. It would appear therefore from this result that the evolution of a 'new' race is not dependent on the interaction of the parasite and a resistant host and that the capacity of such a race to infect a resistant variety is not lost by passage through a susceptible

variety. On the other hand there is evidence in the results published by Hickman and English(38) to suggest that adaptation may occur. One of the isolates they obtained from diseased Auchincruive Climax plants (R.6) proved to be non-pathogenic when Auchincruive Climax plants were inoculated with it. They observed that the diseased plants from which the isolate had been obtained were not so severely infected as other plants of this variety from different localities. It may be that the process of adaptation had not been carried very far when the fungus was cultured and in the limited number of plants inoculated under laboratory conditions infection did not occur.

However physiologic races arise, the fact that they have been proved to occur in yet one more of the parasites which cause serious losses in an economic crop, has made the task of the plant breeder still more difficult. Since there is no reason to suppose that further races will not be found which will be capable of infecting resistant varieties of the future, the prospect of effectively controlling red core disease is bleak indeed. In order to be certain of the complete resistance of a new variety it would be necessary to find some method of assessing the limits of variation of the parasitic capacity of the pathogen. This would appear to be unavoidably linked with the production of varieties resistant to those races already known and would require an infinite amount of time with the prospect of the variation of the parasite exceeding the variation in resistance

characters available in the strawberry plant. Apart from the method at present employed for other diseases in which the plant breeder keeps just one step ahead of the physiologic races by continuous breeding programmes aimed at supplying resistant varieties at intervals, the only other control measure which might prove successful would be the discovery of a systemic fungicide which would render the plant immune from infection. In a recent paper, Stoddard (40) has claimed that such a chemical has been found in Dithane, D-14. It was found that two concentrations of this substance gave 60% and 100% control. It is too early to say yet whether this substance or others like it will prove effective against the physiologic races of P. fragariae when large scale trials in different localities are carried out.

---

## CONCLUSIONS

1. Physiologic races of Phytophthora fragariae occur. At least three were present in the material tested.
2. The races do not differ in spore size or in their temperature relations.
3. The resistance of Oberschlesien is due to the slower penetration of the fungus in the root as compared with the susceptible variety Huxley.
4. The resistance of the Auchincruive varieties is due to the fact that certain races of P. fragariae are non-pathogenic to them. When infected with a race which is pathogenic they are as severely infected as Huxley.

## Literature cited

(1) ALCOCK,N.L.
1929. A root disease of the strawberry. Gard. Chron. 86.14.

(2) ---------- and Howells,D.V.
1936. The Phytophthora disease of strawberry. Sci. Hort. 4.52.

(3) _____, HOWELLS,D.V.,and FOISTER,C.E.
1930. Strawberry disease in Lanarkshire. Scot. Jour. Agr. 13.242.

(4) ANDERSON,H.W.
1935. Black stele root rot of strawberry. Phytopathology. 25.5.

(5) _____
1942. Red stele root rot of the strawberry. Rev. Appl. Mycol. 21.85.

(6) _____, and COLBY,A.S.
1942. Red stele of strawberry on Pathfinder and Aberdeen. Plant Dis. Reptr. 26. 291.

(7) ANON.
1949. 23rd. Annual Report of Dept. of Scientific and Industrial Research. New Zealand.p.50.

(8) ANON.
1950. Report of the Minister of Agr. for Canada for year ending March 31st. 1950.

(9) BAIN,H.F. and DEMAREE,J.B.
1938. Isolation of the fungus causing the red stele or red core disease of strawberries. Science. 88.151.

(10) _____
1945. Red stele root disease of the strawberry caused by Phytophthora fragariae Hick. Jour. Agr. Res. 70.11.

(11) COLBY,A.S. and BOLL,H.L.
1950. Vermilion, a new strawberry variety. Hort. Abs. 20.2412.

(12) CONNERS,I.L., and SAVILLE,D.B.O.
1947. 25th. Annual Report of the Canadian Plant Disease Survey 1945. Rev. Appl. Mycol.26.9.

(13) _____
1950. 28th. Annual Report of the Canadian Plant Disease Survey 1948. Rev. Appl. Mycol.29.81.

(14) DARROW,G.M. and WALDO,G.F.
1941. Strawberry culture. Western United States. U.S. Dept. Agr. Farmers' Bull.1027.

(15) DEMAREE,J.B. and DARROW,G.M.
1937. The red stele root disease of strawberries in the North Eastern United States. Plant Dis. Reptr.21.394.

(16) _____, and JEFFERS,W.F.
1944. Phytophthora fragariae Hick. and mathods of testing strawberry plants for resistance. Phytopathology.34.991.

(17) FISHER,R.A., and YATES,F.
1943. Statistical tables for biological, agricultural and medical research. Oliver and Boyd Ltd. Edinburgh.

(18)

(18) HICKMAN,C.J.
1939. Contribution to the study of strawberry root rot. Trans. Brit. Mycol. Soc. 23.210.

(19) _____
1940. The red core root disease of the strawberry caused by Phytophthora fragariae n.sp. Jour. Pomol.and Hort. Science.18.89.

(20) _____, and ENGLISH,M.P.
1951. Factors influencing the development of red core in strawberries. Trans. Brit. Mycol. Soc. 34.223.

(21) _____ and ENGLISH,M.P.
1951. The susceptibility of strawberry varieties to red core. Trans.Brit.Mycol.Soc. 34.356.

(22) JEFFERS,W.F.
1943. Further progress in breeding strawberries for resistance to red stele. Rev.Appl.Mycol. 22.488.

(23) _____ and DARROW,G.M.
1942. Promising strawberry crosses resistant to the red stele disease. Rev. Appl. Mycol. 21.421.

(24) _____ and JEHLE,R.A.
1946. A summary of the 1946 strawberry red stele situation in Maryland. Plant Dis. Reptr. 30.279.

(25) KADOW,K.J.
1938. Strawberry diseases in Delaware. Plant Dis. Reptr.22.184.

(26) LEONIAN,L.H.
1925. Physiological studies on the genus Phytophthora. Amer. Jour. Bot.12.444.

(27) _____
1926. The morphology and pathogenicity of some Phytophthora mutations. Phytopathology 16.723.

(28) _____
1934. Identification of Phytophthora species. Agr. Expt. Sta. College of Agr. West Virginia University Bull.262.

(29) O'BRIEN,D.G. and McNAUGHTON,E.J.
1928. The endotrophic mycorrhiza of strawberries and its significance. West Scot. Agr. Coll. Res. Bull. 1.

(30) REID,R.D.
1941. Red core disease of the strawberry. Scot. Jour. Agr. 23.264.

(31) _____
1944. Strawberry red core disease. A progress report(1). Fruitgrower,97.9.

(32) SCOTT,D.H.,JEFFERS,W.F.,DARROW,G.M. and INK,D.P.
1950. Occurrence of strains of the strawberry red stele fungus, Phytophthora fragariae Hick. as shown by differential varietal response. Phytopathology 40.194.

(33) SMITH,H.C.
1951. Red core (Phytophthora fragariae)of strawberries. N.Z. Jour. of Sci. and Technology.33.49.

(34) STODDARD,E.M.
1939. Strawberry red stele appears in Connecticut. Plant Dis. Reptr. 23.249.

(35) _____
1951. A chemotherapeutic control of the strawberry red stele. Phytopathology 41.34.

(36) TEMPLE,C.E.
1940. Red stele rot of strawberry. Rev. Appl. Mycol. 19.550.

(37) TREBUCHET,G.
1951. La selection sanitaire du Fraisier. Rev. Appl. Mycol.30.174.

(38) VALLEAU,W.D.
1939. Red stele of strawberry in Kentucky. Plant Dis. Reptr. 23.153.

(39) WALDO,G.F.,DARROW,G.M.,JEFFERS,W.F., DEMAREE,J.B. and MEADER,E.M.
1948. Breeding strawberries for resistance to red stele root disease. Rev. Appl. Mycol. 27.373.

(40) WARDLAW,C.W.
1926. Lanarkshire strawberry disease. A report for the use of growers. Bot. Dept. Univ. Glasgow.

(41) _____
1927. The strawberry disease in Lanarkshire. Ann. Appl. Biol. 14.197.

(42) _____
1927. Some problems of the Lanarkshire strawberry industry. Scot. Jour. Agr. 10.156.

(43) _____
1928. Lanarkshire strawberry disease. Further observations on its biology. Scot. Jour. Agr. 11.65.

(44) WORMALD,H.
1936. Notes on plant diseases in 1935. East Malling Res. Sta. Ann. Rpt. for 1935.p.144.

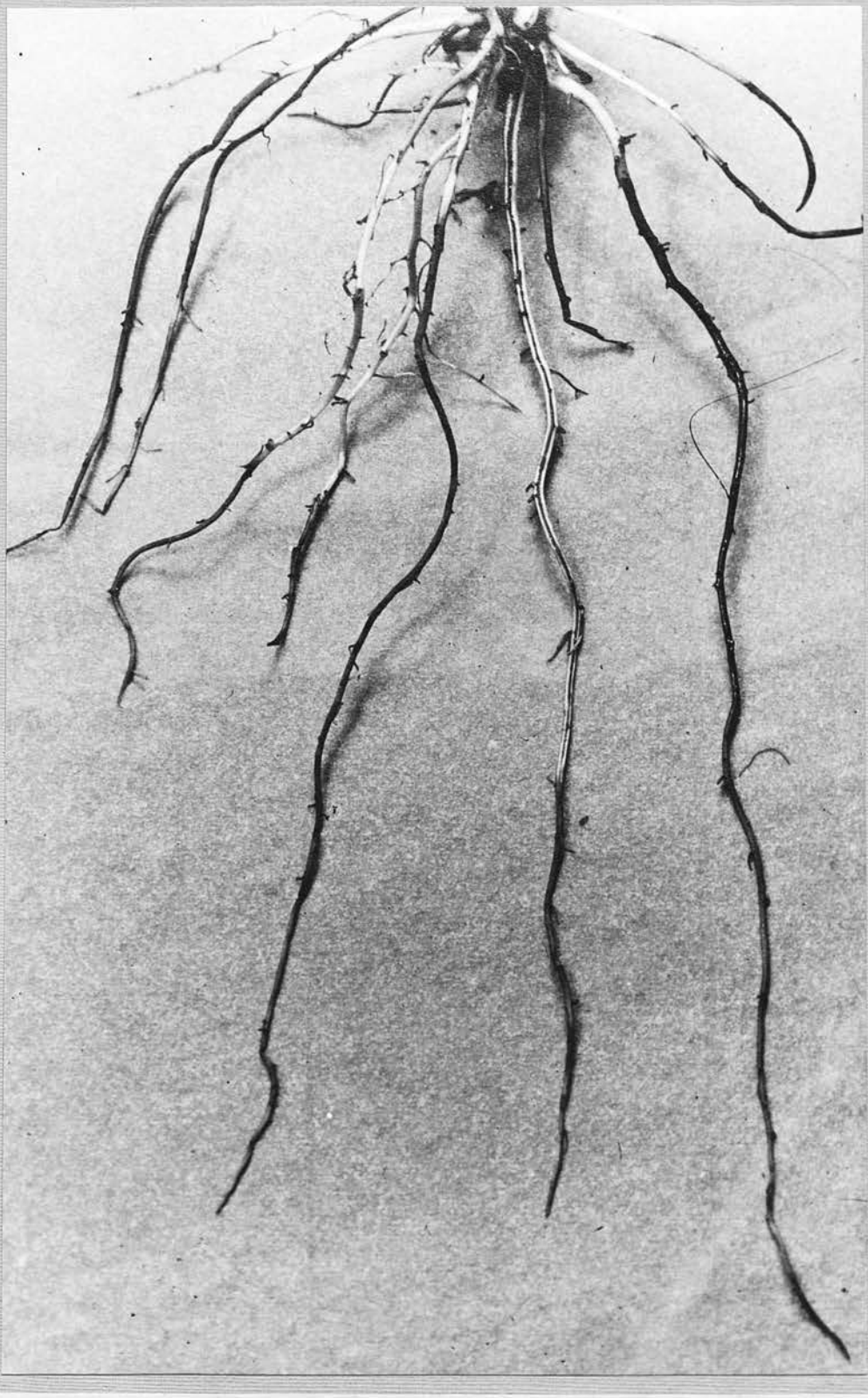


Fig.1. Diseased root system showing absence of fibrous roots and the externally visible rot of the main roots. The rot can be seen extending from the tip backwards towards the rootstock. One of the main roots has been cut open to show the red colouration of the stele extending beyond the rot of the outer tissues.

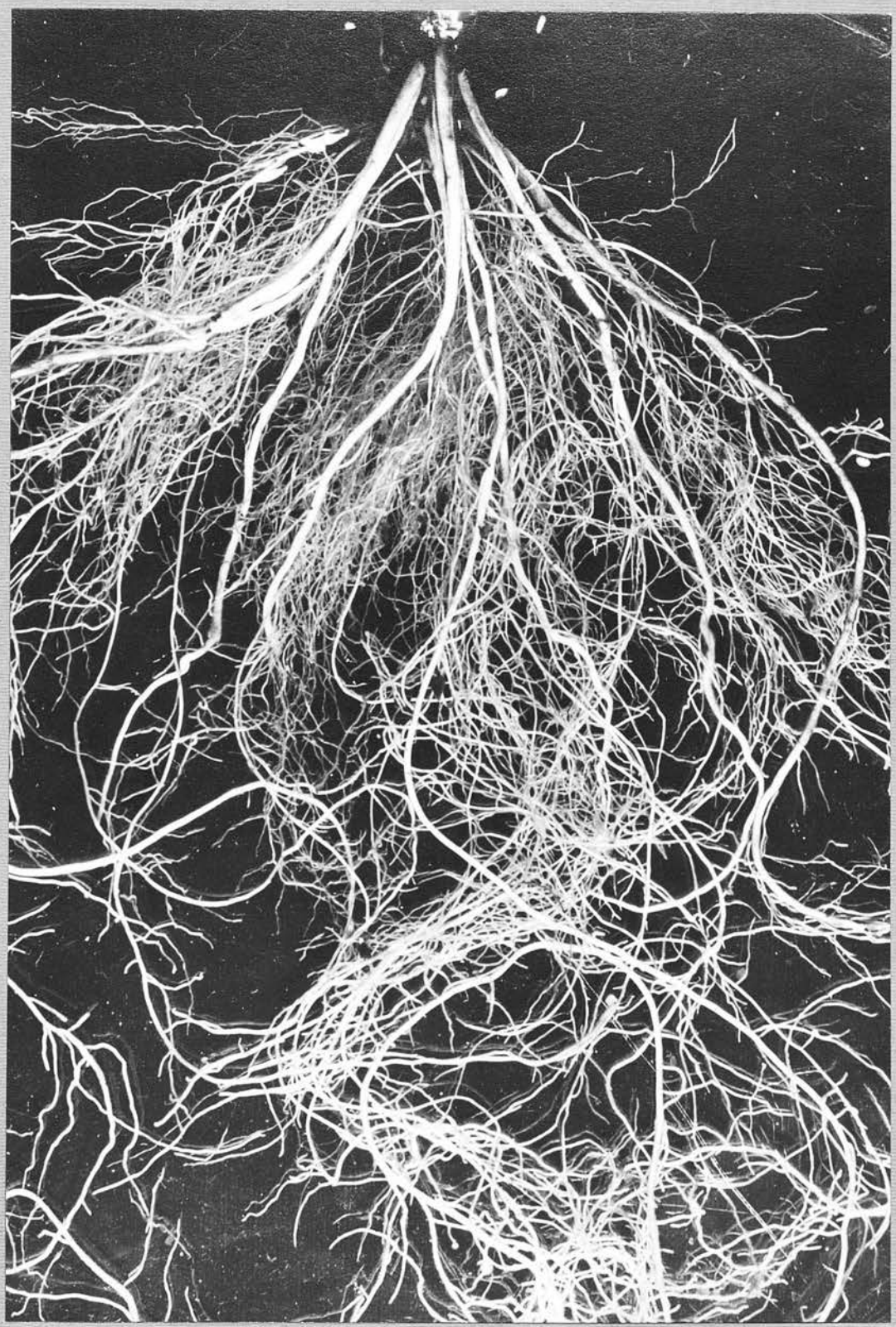


Fig.2. Healthy root system showing fibrous or lateral roots.

Fig.3.

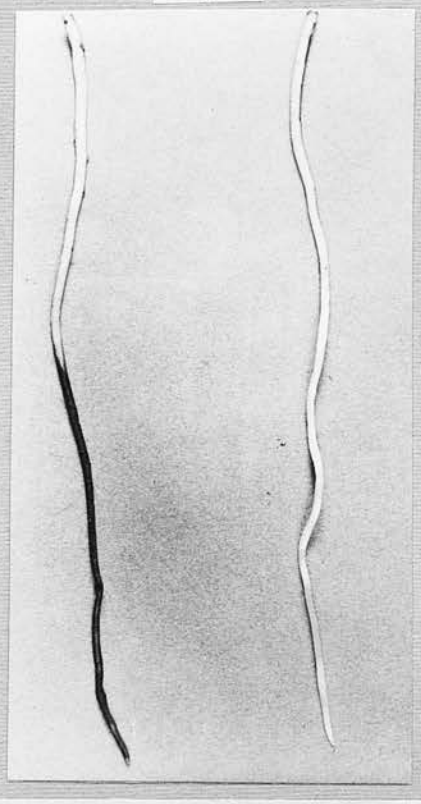


Fig.4.

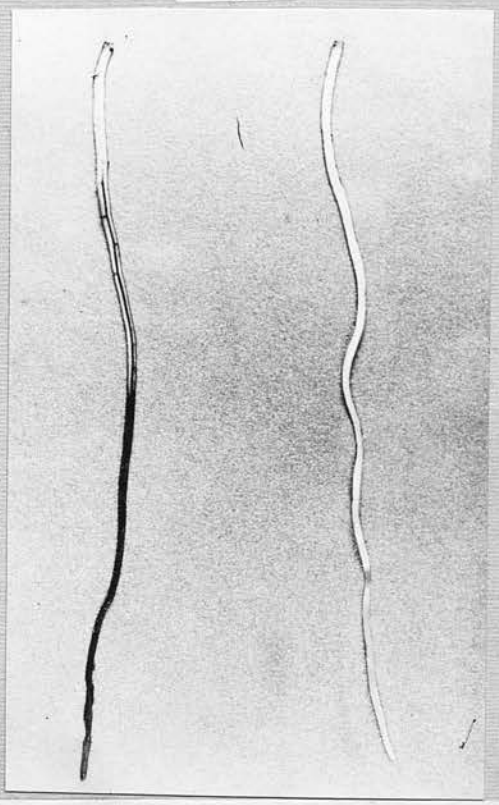
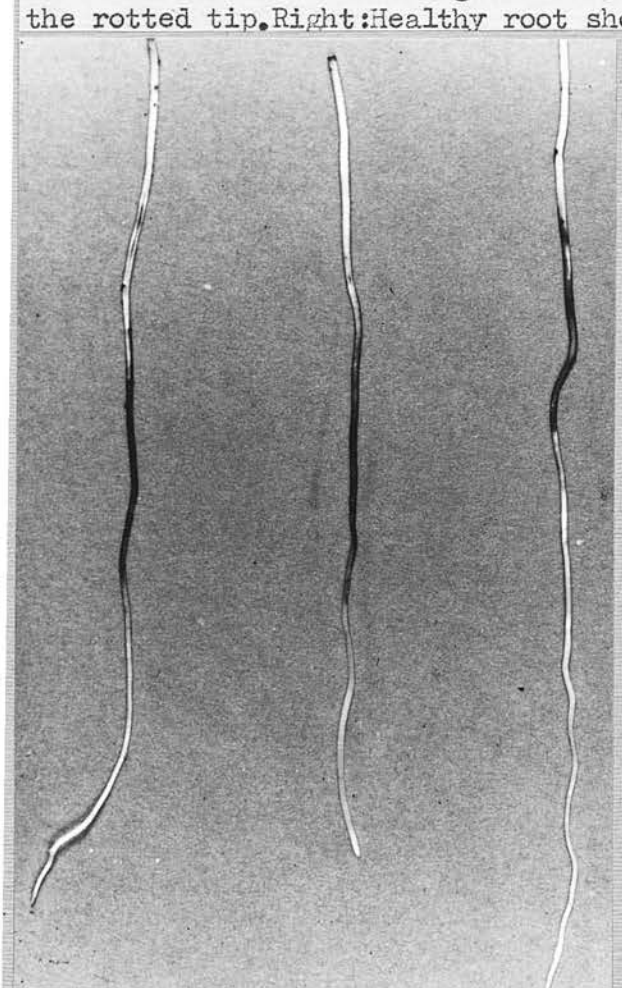


Fig.3.Left: Diseased main root rotting from the tip backwards. Right: Healthy main root. Fig.4. The same roots as in Fig.3 cut longitudinally. Left: Diseased root showing red colouration of the stele extending beyond the rotted tip.Right:Healthy root showing normal white colour of the stele.



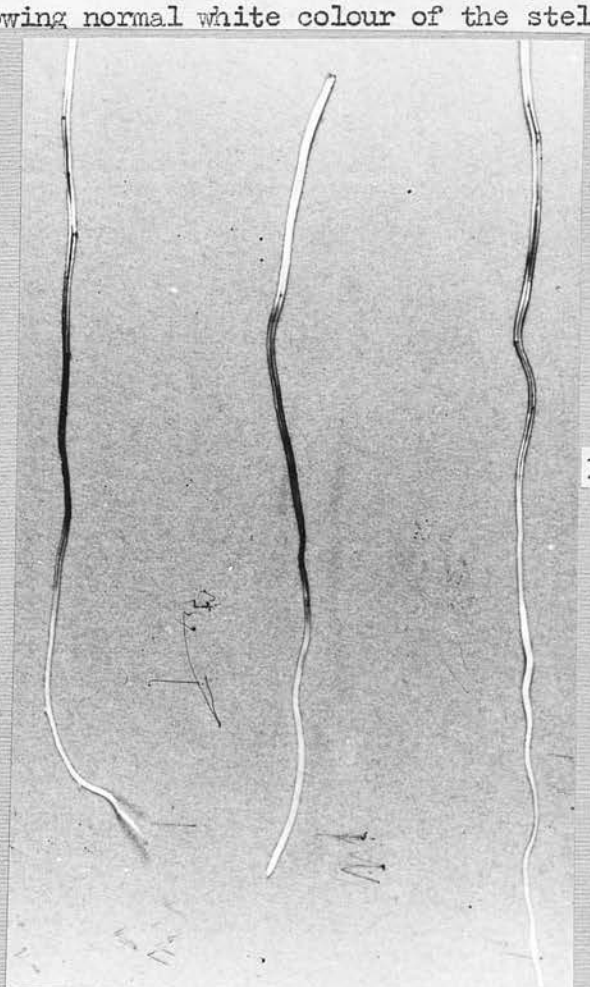


Fig.6


ig.5. Roots attacked by P. fragariae where infection has not proceeded from ıe tip of the root. Note the healthy appearance of the root tips.
ig.6. The same roots as in Fig.5, cut open to show the red colouration xtending from the rotted portions only and no red core at the tips.

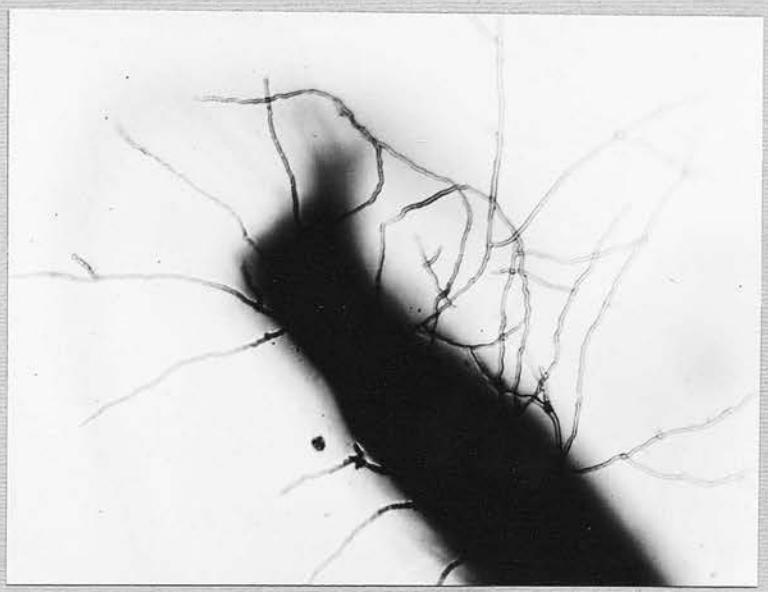


Fig.7. Characteristic non-septate hyphae of P. fragariae growing out from a piece of diseased stele into water agar. Photomicrograph.

Fig.8. Abnormal growth of hyphae of P.fragariae growing from a bean agar disc into malt agar. Photomicrograph.

## II. THE CONDITIONS GOVERNING THE GERMINATION OF THE OOSPORES.

Since it was probable that oospores were the means by which red core disease survived for several years in infected soil which had been free from a strawberry crop during this period, information on the conditions under which they germinated was necessary not only for a better understanding of the life-cycle of the fungus but also for an estimate of the effectiveness of long rotations in the control of the disease in the field.

The oospores of *P. fragariae* are formed abundantly in the region of the stele in all infected roots (Fig.9). They are always contained in a persistent oogonial envelope, irregular but generally oval in shape, with a funnel-shaped base to which the antheridium is attached either in the paragynous (Fig.10) or amphigynous manner. The oogonium is stained a golden brown in colour, the wall and contents of the oospore being colourless. The oospore is usually spherical.

### Review of Literature.

The first account of the germination of an oospore in the genus *Phytophthora* appears to be that by Pethybridge (61) in 1914 when oospores of *P. erythroseptica* were observed to germinate by means of germ tubes or sporangia. Some were observed to have germinated in nine months old cultures on oat extract agar. The internal changes which occurred during germination were studied in hanging drop preparations

in sterilised bog water at room temperature. Pethybridge noted the breaking up of a large central oil drop and the coarsely granular appearance of the protoplasm. He described the thick wall of the oospore as showing "in optical section a series of radial striations which, in surface view, present the appearance of pits of narrow diameter". The inner surface of the wall was observed to be unevenly eroded and to be thinner in oospores which eventually germinated. Pethybridge concluded that the thick wall of the oospore functioned not only as a protection during the resting period but also as a reserve supply of food material which was utilised in the process of germination.

The next contribution to the knowledge of germinating oospores came from Rosenbaum (63) in 1915 when he described a method by which the oospores of P. cactorum could be germinated. The oospore material (sterilised bean pod plugs inoculated with the fungus) was stored in an atmosphere of continuous moisture and buried in soil out-of-doors. After two to three months the oospores were set up for germination, but none occurred. When, however, the material was plunged into sterile distilled water for two to three weeks, oospores germinated within twenty four hours. It is not possible to judge from Rosenbaum's paper whether germination was due to the extra time factor (2-3 weeks), to the submersion in water, or to both of these factors. This work indicated that storage in a moist atmosphere either shortened the maturation period or

was essential for germination to occur since oospores from one year old cultures which had not been stored in this way did not germinate. Rosenbaum's observations on the internal changes which occurred during germination were similar to those of Pethybridge for P. erythroseptica. In P. cactorum the oospore produced a germ tube which eventually bore one or more (proliferating) sporangia. The latter germinated either by a hyphae or zoospores.

In 1918, Murphy (60) described the morphology and cytology of the sexual organs of P. erythroseptica. He demonstrated that the oospore wall was composed of three layers; the primitive wall (the first to be laid down and probably the last to remain after germination), the primary endospore ( a thin hyaline layer) and the secondary endospore ( a thick layer). No exospore was observed by Murphy.

Lafferty and Pethybridge (55) in 1922, described the germination of P. syringae which apparently followed the same lines as P. erythroseptica. In this work oospores were obtained from naturally infected apple tissues so that the age of the spores was probably not known. These authors apparently did not store the oospores for any period before the spores germinated so it is not certain if the oospores of this species require any resting period.

In 1924, Rose (62) observed the germination, by means of a stout branching hypha, of oospores of P. cactorum in old cultures on oatmeal agar but the age of the cultures was not stated. These observations

do not agree with those of Rosenbaum for the same species, since the latter author found that storage in a moist atmosphere was necessary before germination took place and also that the germ tubes bore sporangia of which no mention is made by Rose. However, since Rose did not state either the age of the cultures or the conditions of storage it is possible that the conditions found by Rosenbaum to be necessary during maturation were met with during the ageing of the cultures described by Rose and that the germ tubes did not produce sporangia owing to different external conditions (e.g. higher temperature) during germination.

Blackwell and Waterhouse (51) in 1931 published an account of their observations on the germination of oospores of P. cactorum which they found germinating 'in situ' in cultures on Quaker oat agar six to twelve months old which had been kept continuously moist at 23°C, thus confirming Rosenbaum's finding that moisture was necessary during maturation. Oospores younger than six months old did not germinate. Blackwell and Waterhouse concluded from their work that maturation was the main factor in germination. The oospores were observed to germinate by producing one or two germ tubes one of which usually bore a sporangium.

Blackwell (47) in 1934 ennumerated the facts which were known about the substances in the structure of the oospore, pointing out that it was on the outer almost impermeable membrane of the spore that the

various chemical and physical agencies, which had been used to induce germination of resistant fungal spores, acted. She considered that before germination could take place, this membrane had to be rendered permeable. Since it had been shown that oospores of P. cactorum germinated 'in situ' after a period of maturation alone, Blackwell suggested that the carbon dioxide, accumulating within a resting spore might eventually reach a concentration such that an electric charge was given to the colloids of the impermeable membrane thus rendering it permeable from within.

McKay (56) in 1935 gave the first account of the germination of oospores of Peronospora schleideniana. These spores evidently required an even longer maturation period than those of the Phytophthora spp. so far mentioned. McKay allowed the oospore-bearing material to weather out-of-doors and only after four years were occasional oospores found to germinate when they were placed in water in a warm room. In this instance too a thinning down of the oospore wall was observed prior to germination.

Blackwell (48) in 1935 obtained the germination in large numbers of oospores of Phytophthora cactorum by exposing three months old oospores to a temperature of $1^{o}$- $3^{o}$C for a month and then soaking the spores in water (constantly renewed) for 1-2 weeks. Blackwell emphasised that germination could not take place until the oospore had fully matured (and this she considered to involve a time factor)

and until the outer wall was rendered sufficiently permeable to admit water and oxygen.

Wolf, McLean, and Dixon (65) in 1936 investigated the environmental factors governing the germination of the oospores of Peronospora tabacina. They found occasional germination occurring when oospores had been subjected to intermittent heat and cold for six months. The periods and temperatures were not stated. These investigators studied germination in two lots of material one of which had been air-dried and the other kept in a moist atmosphere. The range of temperature used for germination was 36°-75°F but owing to the very low numbers of oospores which germinated, they did not find it possible to assess the effect of the various factors.

McKay (57) in 1937, discovered that although 2% germination was the highest obtained with four year old oospores of P. schleideniana when they were placed in water, he obtained 60-85% germination in five to six year old spores which had been placed in water containing 0.01-0.02% potassium permanganate solution. The percentage occurring in the controls was 0.05-1. McKay observed that the reagent did not act directly on the oospores since it had no effect if the latter were mounted free from organic remains. The treatment had also no effect on eighteen months old oospores thus confirming Blackwell's observation that there is a time factor as well as a maturation period necessary before germination takes place. In a later paper , McKay (58) reported that the

germination of six to twelve months old oospores of Phytophthora erythroseptica had been observed in water containing potassium permanganate solution but did not indicate whether he had obtained a higher percentage of germination by this method.

Blackwell (49) in 1937 described the germination of the resistant spores of Blastocladia pringsheimii and suggested that the failure of attempts to germinate these spores previously had been due to the use of immature or desiccated spores. She described a method of embedding spore-bearing material in agar in which they remained viable for a period of four to five months at laboratory temperature.

McKay (59) in 1939, whilst working with Peronospora schleideniana observed that the presence of the oogonium was not a necessary factor in germination as oospores which had become free of this envelope germinated in a manner similar to those still contained in it. McKay also noted that thinning of the oospore wall and internal changes went on together but that the latter appeared to be the more important. With seven year old oospores he obtained 5 % germination in water and 95 % when potassium permanganate was added. An increase in germination was also obtained by adding traces of hydrogen peroxide to the water. McKay concluded from these results that once the internal changes which occur inside the oospore reach a certain stage, oxygen is the additional factor necessary for germination.

Blackwell (50) in 1943, traced the development

of the oospore of Phytophthora cactorum from formation to germination. The oospore wall of this species she described as two layered; the exospore (a thin layer highly resistant and almost impermeable) and the endospore (a thick layer ). She observed that the first stage in dormancy was the delay in fusion of the two nuclei which she found to take place three to four weeks after the formation of the oospore. The second stage in dormancy took six to seven months at laboratory temperature but this could be speeded up to one to two months using the low temperature method of storage already described. After this second dormant period, Blackwell found that if the oospores were not germinated, they entered a third period of dormancy which might extend for many months. She noted a difference in appearance between dead oospores and those which were still living. The former were described as looking "bright, hard and glassy" and the latter when prepared for germination as having a "characteristic dull, soft, granular appearance". Blackwell observed that immature oospores lay loosely within the oogonial envelope while matured oospores had expanded almost filling the oogonial cavity. She also found that oospores which showed all indications of being ready to germinate, in that they were thin-walled, multinucleate, and had reserves absorbed into the protoplasm, did not always germinate and concluded that since each oospore was an independent unit, there was probably no set of conditions which would bring about the germination of all oospores at the same time.

Bain and Demaree (45) in 1945, published the first and only account of the germination of the oospore of P. fragariae. They observed this to occur in only one instance when oospores had been placed in hanging drop water cultures and stored at 10°C for six months. The oospore had germinated sometime previously to produce a "long-stalked sporangium" which had discharged its contents and been followed by another sporangium (proliferous) which also appeared to have discharged zoospores in a normal manner. Bain and Demaree observed that roots which had been infected during the autumn, winter and spring period contained a high proportion of normal appearing oospores throughout the succeeding summer. When such roots were examined in the autumn, it was found that the proportion of normal appearing oospores had decreased presumably as a result of germination and by the following spring, although most of these old roots had rotted away, the few that remained contained only a few normal appearing oospores. Bain and Demaree concluded from these observations that the oospores required a resting period before germination could take place and that since summer temperatures in the U.S.A. were undoubtedly high enough to kill the mycelial stage of the disease, these spores served to over-summer rather than to over-winter red core in that country. They thought it unlikely that P. fragariae could maintain itself in the soil if strawberry plants were not available at the critical period when the oospores were germinating and that the longevity of

the oospore might well determine the length of time the disease could exist in land which was kept free from strawberry plants.

Drechsler (53) in 1947 obtained the germination of oospores of Pythium aphanidermatum derived from sixty day old maize meal agar cultures, by transferring them to a shallow layer of distilled water. Here too the absorption of the thick inner layer of the oospore wall was observed.

Barrett (46) in 1948 mentioned the germination of oospores of Phytophthora drechsleri but gave no information on the conditions under which germination occurred.

Cheo and Leach (52) in 1950 described a method by which they had obtained the germination of spores of Ustilago striiformis. This consisted of soaking the spore mass for fifteen days or longer in a concentrated dung infusion, then dispersing it in distilled water in which germination took place.

Gimesi and Frenyo (54) found that the lack of germination of Tilletia spores was associated with an inhibitor which was removed by adsorption when the spores came into contact with soil, especially humus, and that germination then proceeded freely.

## METHODS.

In order to investigate the effect of various treatments on the germination of oospores, it was necessary to have large numbers of these spores in such a condition that microscopic observations could be made. In preliminary tests, the cortex was removed

and the oospores dissected out of the tissues of the stele by means of fine needles under a dissecting microscope. In this way, large numbers of oospores almost entirely free from root tissues were obtained, but the oogonial envelope proved to be very persistent and in only a few cases had the envelopes burst to release the colourless oospores. The latter were ideal for observational purposes as the deep golden brown colour of the oogonial envelope made it impossible to see clearly the progressive internal changes leading to germination. However, as three hundred oospores were considered the minimum number necessary for each treatment in order to obtain reliable results, this method which was extremely laborious proved to be impracticable. In an attempt to find a quicker method, large numbers of infected roots were placed in an Ato-Mix with as small a volume of water as was possible. The resulting maceration of the tissues was not sufficiently great to separate many of the oospores from the tissues of the stele and they were accompanied by such a large volume of root tissues that no advantage was obtained by this method.

The method eventually adopted was to tease out the tissues of the stele after removing the cortex. This was done with fine dissecting needles without the aid of a dissecting microscope. A number of oospores always remained deeply embedded in the tissues of the stele but the majority were either free or attached to the stele in such a way that they

could be observed quite clearly. In assessing the number of oospores for each treatment only those which could be clearly seen were counted. Thus any oospores which were buried in the tissues of the stele and later germinated were not counted in calculating the percentage of germination. Three hundred oospores were used for each treatment described below.

When oospores were set up for germination, the glass slides on which pieces of stele had been teased out were placed in large Petri dishes containing damp filter paper which maintained a high humidity so preventing the slides from drying out. The Petri dishes were placed at 20°C and after forty eight hours, cover-slips were placed over the preparations and final observations made.

TREATMENTS.

The first series of experiments was designed to test the capacity of various conditions and reagents for inducing germination of oospores which had not undergone any period of maturation or to confirm the findings of previous workers that this resting period was necessary before germination could take place. The oospores were therefore obtained from recently infected roots. The treatments which were used are listed in Table 11.

No germination occurred in any of these treatments or in the control. The only reagent which produced a change in the oospore was Hortomone A at the lower dilution (d,i). In this hormone preparation the oospores enlarged considerably, the oogonial

envelopes split open and many of the oospores showed narrow cracks extending across the thickness of the endospore wall, through which the protoplasmic contents were extruded as a broad ribbon. The enlargement of

Table 11. No period of maturation.

| Treatments | |
|---|---|
| (a) | Roots containing oospores were placed alongside actively growing healthy roots of a susceptible variety. After four days at a temperature of 15 -18°C they were removed and the oospores set up in water. |
| (b) | Juice extracted by pressure from healthy strawberry roots. |
| (c) | Dung infusion (i) concentrated<br>(ii) diluted with water (1:1) |
| (d) | Hortomone A (i) 1 pellet dissolved in four pints of water<br>(ii) 1 pellet dissolved in eight pints of water |
| (e) | Vitamin $B_1$ (50 mg. in 25 cc. water) |
| (f) | $\beta$- Indolyl acetic acid (20 mg. in 10 cc abs. alcohol) |
| Control | Tap water |

the oospore and the subsequent cracking of both the oospore and oogonial wall was presumably due to the abnormal absorption of water by the protoplasm of the oospore. The hormone evidently acted on the wall of the oospore rendering it permeable so that absorption of water could take place but there appeared to be another effect in that absorption proceeded beyond the point normally reached by a living spore. This suggested that this hormone preparation had acted on the protoplasm of the oospore in such a way as to increase considerably its water holding capacity but

how this was brought about cannot be explained.

The results obtained with the various treatments tabulated in Table 11, indicated that none of the reagents used were capable of inducing germination of immature oospores and that a maturation period was necessary before germination could take place.

The next series of treatments was aimed at assessing the effect on germination of maturation under different conditions in the laboratory. Again, newly infected roots were used so that the oospores had no opportunity of maturing under natural conditions. After the various treatments, details of which are given in Table 12, each sample of three hundred oospores was halved, one portion being set up in tap water to which had been added a few drops of a 0.01-0.02 % solution of potassium permanganate, the other being placed in tap water alone.

Table 12. Artificial maturation.

| Period (months) | Conditions |
|---|---|
| 5 | 1)a. Culture of bacteria derived from strawberry roots added to shallow layer of water in Petri dish. Temperature 20°C<br>b. Shallow layer of water in Petri dish. Temperature 20°C |
| 4 | 2)a. Shallow layer of water in Petri dish. Temperature 1°-6°C.<br>b. Damp sphagnum moss. Temperature 1-6°C. |
| 2 | 3) Shallow layer of water in Petri dish. Alternating temp. 20°C(12 hours)<br>1°-6°C(12 hours) |
| $5\frac{1}{2}$ | 4) Shallow layer of water. Alternating temp. 20°C($1\frac{1}{2}$ months) 0°-5°C(4 months) |
| 1<br>2<br>3<br>4 | 5)a. Alternating wet and dry. Temp. 5°-<br>b. 12°C.<br>c.<br>d. |

No germination was observed after any of these treatments. It is not understood why treatments which included maturation periods of over three months did not result in germination since, as will be seen later, naturally matured oospores germinated after this period. As far as is known the roots containing the oospores were in no instance deprived of oxygen or subjected to any other factor which could have caused the death of the oospores. Also, none of the temperatures used were very different from those to which naturally maturing oospores would be subjected in the soil. Treatment 2)a. in Table 12 is similar to the condition of refrigeration recommended by Blackwell (48), although the oospores the latter used were three months old before refrigeration commenced and were soaked in water for two to three weeks before being set up for germination. Failure of oospores to germinate as a result of treatment 2)a. might have been due to the absence of either or both of these factors. If it is assumed that some factor, or factors necessary for germination were missing from these treatments it must be concluded that they were not the factors of maturation, moisture, temperature and oxygen employed here or that they involve different combinations of these conditions.

In the third series of treatments, the results of which are shown in Table 13, natural maturation of the oospores was allowed to take place. A large mass of recently infected roots was divided into two and stored in the following way. One half was placed in a wooden box sunk into soil outside where no strawberry

plants were growing. The other half was tied up in a muslin bag which was then buried in the soil of a large porous pot containing a healthy strawberry plant of the susceptible variety Royal Sovereign. The pot was then plunged into the ground so that the soil within the pot was at the same temperature and moisture content as the surrounding soil. At one month intervals, samples were taken from each lot of roots and the percentage of oospores which germinated within forty eight hours was calculated.

Table 13. Percentage of germination as a result of natural maturation.

| Period of maturation (months) and month sampled. | Type of storage | |
|---|---|---|
| | Wooden box sunk in soil outside. No strawberry plants growing in the vicinity. A | Muslin bag buried in soil of porous pot containing healthy Royal Sovereign plant. B |
| October 1 | 0 | 0 |
| November 2 | 0 | 0 |
| December 3 | 0 | 0 |
| January 4 | 1 | 3.6 |
| February 5 | 4.6 | 0 |
| March 6 | 1 | 3.3 |
| April 7 | 0 | 0.3 |
| June 9 | 0 | 0 |

Both potassium permanganate at the dilution

recommended by McKay (57) and hydrogen peroxide were added to the water containing oospores from both treatments at various times but although similar percentages of germination were recorded in potassium permanganate solution as in water alone no increase in the figure of germination was obtained in this solution. In hydrogen peroxide solution no germination was obtained.

Although the percentage of germination in both treatments was extremely small, there did not appear to be any appreciable increase when the oospores were in the vicinity of growing strawberry roots. It would seem therefore that there would be no influence exerted by a crop of strawberry plants in the field on the germination of oospores already in the soil and that the latter would germinate at the same rate in land which was kept free from strawberry plants.

In the limited number of samples taken there appeared to be no increase in the percentage of germination with longer periods of maturity. Evidently after the minimum period of maturation, which in the case of P. fragariae is probably between three to four months, the oospores germinate a small number at a time throughout the period of their longevity.

In treatment A after five months maturation, when the material was being dissected, it was observed that a number of oospores were in the process of germinating. This would indicate that germination was occurring in the soil at this time of the year and did not occur only as a result of transferring the

oospores to drops of water at 20°C.

The extremely low rate of germination confirms Blackwell's (50) findings that all oospores did not germinate at the same time and this is no doubt the reason for crops becoming infected on land which has been free of strawberries for a number of years. Presumably there would always be at least a few oospores germinating at the time when the susceptible host plant is being grown.

All the oospores which germinated produced a germ tube which eventually bore a single sporangium similar in shape to those produced by mycelium (Fig.11). The sporangium at maturity was cut off from the germ tube by a thick plug and was observed to germinate in the usual manner by the liberation of at least eight zoospores (Fig.12). Owing to the rarity of germination it was not possible to study the internal changes which occurred prior to germination. In material which was neither fixed nor stained, the dark colour of the oogonial envelope which was closely adhering to all germinated oospores obscured the character of the contents of the oospore. It was possible however to observe that the thick wall of the oospore had disappeared during the process of germination.

## DISCUSSION.

The most important condition governing germination is undoubtedly maturation and there seems to be no factor other than time which can bring this about. It has been shown that an interval of about four

months in soil is sufficient in itself for germination to occur but as all attempts to reproduce similar conditions of temperature and moisture together with the same interval of time resulted in failure of any oospores to germinate, it has not been possible to determine what factors other than time are necessary.

Until it has been proved that P. fragariae is capable of leading a saprophytic existence in the soil the effectiveness of long rotations on the control of red core will obviously depend on the longevity of the oospore. This has not been determined experimentally but there is some evidence that this may be as long as fifteen years.

## CONCLUSIONS

1. A minimum period of three to four months maturation is necessary for germination to occur.
2. Factors other than time, temperature and moisture are probably involved in the maturation of the oospore.
3. The amount of germination which occurs is not influenced by the presence of actively growing strawberry roots in the vicinity.

## Literature cited.

(45) BAIN,H.F. and DEMAREE,J.B.
1945. Red stele root disease of the strawberry caused by Phytophthora fragariae Hick. Jour. Agr. Res. 70.11.

(46) BARRETT,J.T.
1948. Some conditions contributing to the development and germination of oospores in the genus Phytophthora. Phytopathology.38.913.

(47) BLACKWELL,E.M.
1934. The germination of oospores of Phytophthora cactorum. Trans. Brit. Mycol. Soc. 19.157.

(48) _____
1935. Germination of resting fungal spores. Nature 135. 546.

(49) _____
1937. Germination of resistant spores of Blastocladia pringsheimii. Nature 140.933.

(50) _____
1943. The life-history of Phytophthora cactorum (Leb.& Cohn)Schroet. Trans. Brit. Mycol. Soc. 26.71.

(51) _____ and WATERHOUSE,G.M.
1931. Spores and spore germination in the genus Phytophthora. Trans.Brit.Mycol. Soc. 15.294.

(52) CHEO,P-C, and LEACH,J.G.
1950. The stimulating effect of dung infusion on the germination of spores of Ustilago striiformis. Phytopathology 40.584.

(53) DRECHSLER,C.
1947. Germination of oospores of Pythium butleri and Pythium tardicrescens. Phytopathology 37.438.

(54) GIMESI,N. and FRENYO,V.
1950. The basis of the stimulating effect of humus on the germination of Tilletia spores. Biol. Abs. 24. 2665.

(55) LAFFERTY,H.A. and PETHYBRIDGE,G.H.
1922. On a Phytophthora parasitic on apples which has both amphigynous and paragynous antheridia; and on allied species which show the same phenomenon. Sci.Proc.Roy.Dublin Soc. 17(n.s.).29.

(56) McKAY,R.
1935. Germination of resting spores of onion mildew (Peronospora schleideni). Nature 135. 306.

(57) _____
1937. Germination of oospores of onion mildew, Peronospora schleideniana W.G.Sm. Nature 139.758.

(58) McKAY,R.
1937. Potassium permanganate as an aid to the production of asexual fructifications by Phytophthora erythroseptica Pethyb. Nature 139.802.

(59) _____
1939. Observations on onion mildew caused by the fungus Peronospora schleideniana. Jour. Roy. Hort. Soc. 64.272.

(60) MURPHY,P.A.
1918. The morphology and cytology of the sexual organs of Phytophthora erythroseptica Pethyb. Ann.Bot.32.115.

(61) PETHYBRIDGE,G.H.
1914. Further observations on Phytophthora erythroseptica Pethyb. and on the disease produced by it in the potato plant. Sci.Proc.Roy. Dublin Soc.14(n.s) 179.

(62) ROSE,D.H.
1924. Leather rot of strawberries. Jour.Agr.Res. 28.357.

(63) ROSENBAUM,J.
1915. Phytophthora disease of ginseng. N.Y. Cornell Univ. Agr.Exp.Sta.Bull.363.

(64) _____
1917. Studies of the genus Phytophthora. Jour. Agr. Res. 8. 233.

(65) WOLF,F.A.,McLEAN,R.A.,and DIXON,L.F.
1936. Further studies on downy mildew of tobacco.Phytopathology 26.760.

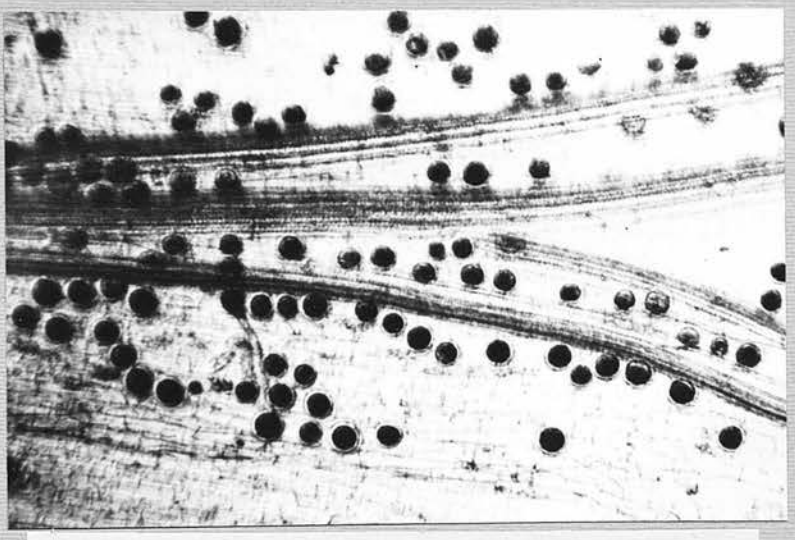


Fig.9. Oospores of P. fragariae in squashed root preparation mounted in lactophenol and cotton-blue. Photomicrograph. 120.

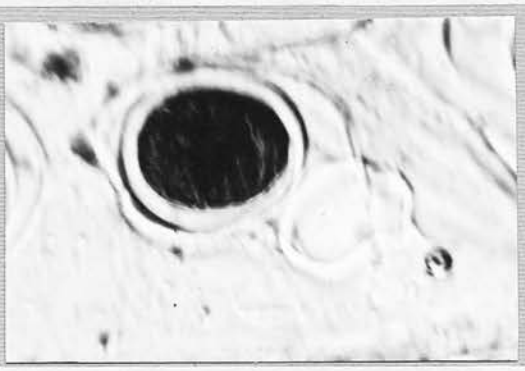


Fig.10. Oospores of P. fragariae lying within the oogonial envelope which has a paragynous antheridium attached to the base. Note the thick wall of the oospore. Mounted in lactophenol and cotton-blue. Photomicrograph. 700.

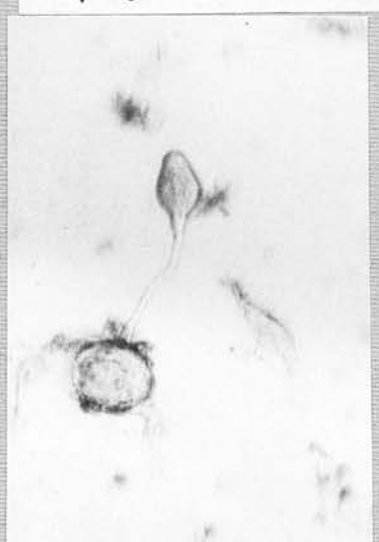


Fig.11.

Germinated oospore showing germ tube bearing a sporangium. Mounted in water. Photomicrograph. 300.

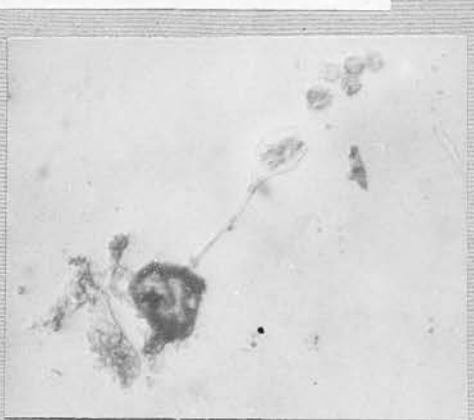


Fig.12.

The same oospore as in Fig.11 eight hours later. The sporangium has germinated by the production of at least eight zoospores, five of which may be seen just beyond the sporangium and three of which remained in the sporangium. Photomicrograph. 300.

## Summary

The occurrence of physiologic races of *Phytophthora fragariae* is demonstrated. A comparison is made of the size of sporangia, zoospores and oospores produced by them, and the temperature relations of the races are determined.

The differential response to red core of five varieties of strawberry, namely, Huxley, Oberschlesien, Auchincruive 6, Auchincruive Climax and Auchincruive 11 is studied.

The conditions governing the germination of the oospores are investigated.